DISTRIBUTION AND ELIMINATION OF LOCAL ANAESTHETIC AGENTS: THE ROLE OF LUNG, LIVER AND KIDNEYS

BY

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DECLARATION

I declare that this thesis is of my own composition and is a record of my own research, not having been presented in any previous application for a degree. Help given by others is acknowledged overleaf and all sources of information are indicated in the text.

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CONTENTS

SECTION 5

PHARMACOKINETICS OF PRILOCAINE IN NORMAL AND ANEPHRIC ANAESTHETISED RABBITS

SECTION 6

LUNG UPTAKE OF LIGNOCAINE AND PRILOCAINE IN ANAESTHETISED HUMAN SUBJECTS

ABSTRACT

A pharmacokinetic study of prilocaine hydrochloride in human volunteers was performed. The total body clearance of this drug was found to be greatly in excess of values reported for other amide type local anaesthetics and also to be in excess of human liver blood flow. An extra-hepatic site for prilocaine metabolism has been proposed and the possible site of this metabolism was investigated.

In vitro incubation experiments using lung and kidney slices from dogs and rabbits were performed. The results confirmed other reports that both tissues were capable of metabolising prilocaine, and in greater quantities than lignocaine.

Experiments with isolated perfused rat lung preparations showed that after equilibration with ^{3}H lignocaine and ^{3}H prilocaine in the perfusion medium, prilocaine was removed from the perfusate to a greater extent than lignocaine. In further experiments with isolated perfused rat lungs, utilising a single pass bolus injection technique, prilocaine was retained in the lung tissue to a greater degree than lignocaine.

The pharmacokinetics of prilocaine in anaesthetised dogs was investigated. After infusing prilocaine to steady state blood concentrations, simultaneous sampling of blood from the pulmonary artery, through a Swan-Ganz

catheter, and from the aorta was undertaken. Drug analysis of these samples showed a small decrease in prilocaine concentrations from the pulmonary artery to the aorta in two preparations. Total body clearance of prilocaine in dogs was found to equal hepatic blood flow. The validity of the pharmacokinetic model used for analysis was tested using computer predicted drug concentration data and found to be correct. Direct determination of the hepatic extraction of prilocaine in dogs confirmed that hepatic metabolism was not totally responsible for the clearance of this drug.

The pharmacokinetics of prilocaine in anaesthetised rabbits were also investigated and again, total body clearance of the drug was found to equal hepatic blood flow. A similar study with anaesthetised anephric rabbits showed both an increase and decrease in clearance in different preparations. These differing results have been attributed to surgical trauma.

Experiments to observe the first pass buffering effect of the lung on a bolus injection of a prilocaine and lignocaine mixture in anaesthetised human subjects was undertaken. The human lung was shown to markedly reduce the possible peak concentrations of both drugs, prilocaine concentrations being reduced to a greater degree than lignocaine concentrations.

The implications of these results with respect to distribution, metabolism and toxicity of amide type local anaesthetics have been discussed.

INTRODUCTION

INTRODUCTION

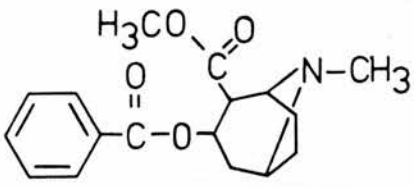
The concept of the use of local anaesthetics in clinical practice is generally attributed to Koller (1884) who used cocaine (Fig 1) for topical anaesthesia of the eye. New techniques in local anaesthesia followed and by 1900 cocaine had been used for peripheral nerve blockade and spinal anaesthesia. The occurrence of severe toxic side effects and the addictive nature of this drug led to the search for synthetic compounds with local anaesthetic properties to replace cocaine.

The development of benzoic acid ester derivatives in the early 1900s, eg procaine and benzocaine (Fig 1), was a major step forward in improving the safety and clinical usefulness of local anaesthetics. However, a major disadvantage of these drugs was their poor chemical stability in solution. These compounds were also capable of producing unwanted side effects and generally had a short duration of activity.

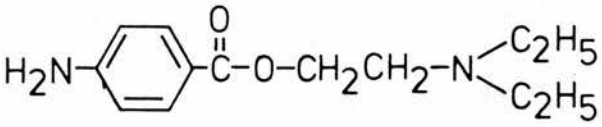
In 1943, Löfgren developed lignocaine (Fig 1), an amide derivative of diethylamino acetic acid. Although preceded by the introduction of cinchocaine (Fig 1), a quinoline derivative, the development of lignocaine was to lead to the production of a new family of amide type local anaesthetics (Fig 1). These drugs proved to be more stable than the ester type local anaesthetics and generally had a longer duration of activity.

FIGURE 1

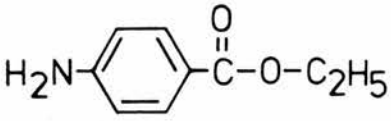
CHEMICAL STRUCTURES OF SOME LOCAL ANAESTHETIC AGENTS



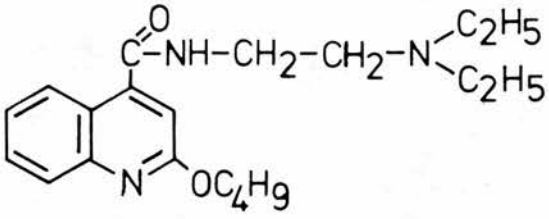
COCAINE



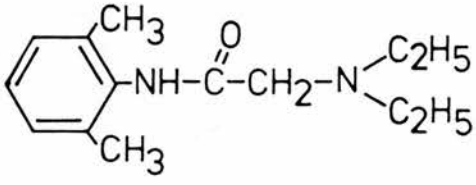
PROCAINE



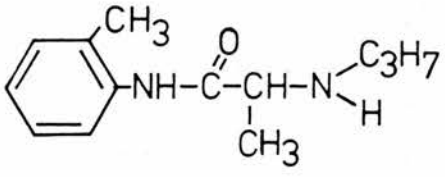
BENZOCAINE



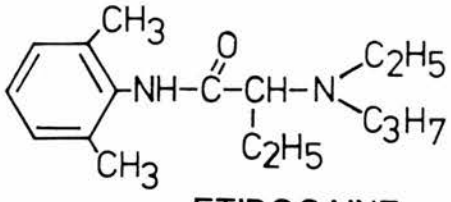
CINCHOCAINE



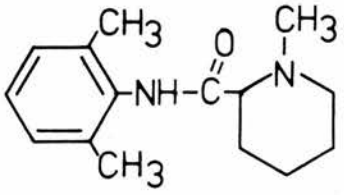
LIGNOCAINE



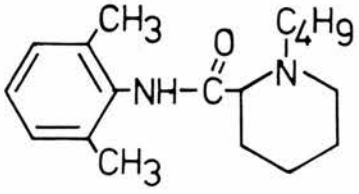
PRILOCAINE



ETIDOCAINE



MEPIVACAINE



BUPIVACAINE

Prilocaine, synthesized by Löfgren & Tegnér (1960) was found to have local anaesthetic qualities similar to those of lignocaine but to have a lower toxicity (Weidling, 1960; Åström & Persson, 1961). The main structural differences between prilocaine and lignocaine are that prilocaine is a secondary amine whereas lignocaine is a tertiary amine, and lignocaine is a xylidine derivative whereas prilocaine is a toluidine derivative. Mepivacaine and bupivacaine are characterised by their amine group being incorporated into a ring system and etidocaine by its ethyl group on the intermediate chain of the molecule.

This group of compounds display different local anaesthetic properties and therefore they have different clinical applications. Prilocaine, lignocaine and mepivacaine are generally classified as being local anaesthetics of intermediate potency and bupivacaine and etidocaine as local anaesthetics of high potency. The onset of anaesthesia tends to occur more rapidly with the intermediate potency drugs than with the high potency drugs but the latter group usually exhibit a longer duration of action (Covino & Vassallo, 1976).

There are several factors which affect both the time to onset and the duration of local anaesthesia. With subcutaneous infiltration techniques the onset of sensory anaesthesia is almost immediate, whereas in peripheral and central nerve blocks the onset of

anaesthesia may take several minutes, eg 10-15 min for lignocaine and 15-25 min for bupivacaine in epidural anaesthesia (in Lund et al, 1975). The longest latency to onset of anaesthesia occurs with peripheral nerve blocks involving large nerves, eg brachial plexus block, and these differences are related to the speed of drug penetration to the site of action.

An increase in the amount of drug administered tends to reduce the onset time and increase the duration of anaesthesia. The duration of local anaesthesia with these different anaesthetic techniques can depend on the blood flow through the region of anaesthesia, which effectively governs the rate at which the drug is removed from the site of action. The addition of vasoconstrictors to local anaesthetic solutions increases the duration of most nerve blocks; the agent most commonly used being adrenaline in a 1:200,000 dilution. Vasoconstriction decreases the rate at which the circulation removes the local anaesthetic from the site of action and hence drug remains at the site of injection for a longer time.

Of the amide type local anaesthetics, lignocaine has been most extensively investigated as it has not only been used as a local anaesthetic but has also been given intravenously for the treatment of cardiac arrhythmias occurring during myocardial infarction.

Initial studies on the toxicology, mode of action and fate of lignocaine in the body were summarised by

Weidling (1964). The toxic effects of lignocaine and the other amide type local anaesthetics have been well documented (Scott et al, 1973; Usubiaga et al, 1966; Scott, 1975) and reviewed (Fink, 1973; Covino & Vassallo, 1976).

Initial symptoms are subjective, with numbness of the tongue and circumoral numbness occurring due to high tissue concentrations of drugs in these very vascular areas, followed by a feeling of lightheadedness or dizziness, tinnitus and visual disturbances due to mild central nervous system effects. Objective symptoms are slurred speech and muscular twitching. High doses may cause convulsions and eventually respiratory arrest. The resulting hypoxia and acidosis caused by apnoea occur very rapidly during convulsions because the muscles are using large quantities of oxygen (Moore et al, 1980). This leads to myocardial depression followed eventually by cardiac arrest.

These toxic effects occur infrequently in general use of local anaesthetics and usually result from inadvertent intravenous or intra-arterial injection (Tucker & Mather, 1979). For certain purposes local anaesthetics are injected directly into a vein. In Bier's block, for example, the application of an arterial tourniquet is used to stop blood circulation in the limb that is to be anaesthetised, following which local anaesthetic injected intravenously into the limb will

produce anaesthesia as long as the tourniquet is applied. By the time the tourniquet is released, a high proportion of the injected drug will have been absorbed by the limb tissues and only a proportion will be released into the general circulation. However, if the tourniquet is released prematurely, toxic amounts of local anaesthetic may gain access to the circulation. Lignocaine is frequently administered intravenously as a cardiac anti-arrhythmic and, under conditions of reduced cardiac output, toxic concentrations of this drug may occur (Prescott et al, 1976; Thompson et al, 1971).

While intravascular injection is the commonest cause of toxicity, severe reactions do occur occasionally during regional anaesthesia and there are a number of factors which influence this toxicity. The most important are the site of injection, the addition of vaso-constrictors, the acid/base status of the patient, the drug used and the hepatic blood flow. The highest blood concentrations of local anaesthetics are found after injection into highly vascular regions, eg intercostal block. However, adipose tissue at the site of drug administration can absorb large quantities of these local anaesthetics and hence reduce the rate of their release into the circulation, even in relatively highly vascular areas, eg the lumbar epidural space. As previously mentioned, the use of adrenaline as a vasoconstrictor in local anaesthetic solutions causes a decrease in blood flow at the site of injection and this too reduces the

rate of release of local anaesthetic into the circulation.

The physiologic status of a subject is an important factor influencing local anaesthetic toxicity. The acid/base balance has been shown to have marked effects on local anaesthetic blood concentrations (Sjöstrand & Widman, 1973) and acidosis, both metabolic and respiratory, increases the toxicity of these drugs (Englesson, 1974; Englesson & Grevsten, 1974). The amide type local anaesthetics are prepared as hydrochloride salts for clinical use and they have pKa values close to the physiological pH (mepivacaine 7.6, lignocaine 7.7, prilocaine 7.7, etidocaine 7.7, bupivacaine 8.1; from Covino & Vassallo, 1976). Thus, changes in blood pH will result in relatively large alterations in the ratio of uncharged to cationic form of the drug. The form in which these drugs are most readily absorbed through cell membranes is as uncharged base, thus, as blood pH falls, more cationic drug is present in the blood and the amount of drug available to be absorbed by the tissues is reduced.

Englesson (1974) and Englesson & Grevsten (1974) concluded that the increased central nervous system toxicity of local anaesthetics during respiratory acidosis, particularly in the presence of metabolic acidosis, was due to lowering of the pH of brain tissue, which is particularly susceptible to increasing pCO_2 values. This increases the cationic form of drug in the brain and hence maintains the blood/brain tissue base concentration

gradient causing a greater accumulation of local anaesthetic in brain tissue.

The drug used for a local anaesthetic procedure also has some bearing on toxicity. When administered intravenously, prilocaine is the least toxic of the amide group of drugs, followed in order of increasing toxicity by mepivacaine, lignocaine, etidocaine and bupivacaine. Because etidocaine and bupivacaine are both high potency local anaesthetics, smaller amounts of these drugs are used in clinical practice, thus reducing the possibility of toxic reactions occurring. Although prilocaine is the least toxic, its metabolic products have been shown to produce methaemoglobinaemia in man. However, a clinically significant reduction of the oxygen carrying capacity of the blood has only been found after administration of very large doses of this drug (Crawford, 1965; Lund, 1965). This reaction to prilocaine limits its use when pain relief by local anaesthesia involves multiple injections in patients with an already impaired oxygen transport system. The most appropriate procedures for the use of this drug would appear to be those requiring a single dose of less than 600 mg to provide adequate anaesthesia, eg in a comparison of prilocaine (450 mg), lignocaine (450 mg), bupivacaine (150 mg) and etidocaine (150 mg) for brachial plexus block (Wildsmith et al, 1977), the quality and duration of anaesthesia with prilocaine and the low plasma prilocaine concentrations resulting from this technique, led to the conclusion that prilocaine

was the drug of choice for this procedure.

The main site for metabolism of amide type local anaesthetics is the liver and it has been shown that hepatic blood flow is the primary controlling factor in the clearance of these drugs from the body (Tucker et al, 1977). It is apparent that any situation resulting in reduced liver blood flow, eg shock involving a reduced cardiac output, will increase the possibility of a toxic reaction to local anaesthetic administration.

The amide type local anaesthetics are considered to be almost totally metabolised before excretion, with only very small amounts of unchanged drug found in the urine. Values for urinary excretion of unchanged drug have been given as 3% for lignocaine (Keenaghan & Boyes, 1972), less than 1% for prilocaine (Mather, 1972 in Mather & Tucker, 1978), 1% for mepivacaine (Meffin et al, 1973), less than 1% for etidocaine (in Mather & Tucker, 1978) and 1-10% for bupivacaine (in Mather & Tucker, 1978).

The processes involved in the hepatic metabolism of the local anaesthetics have been reviewed (Hansson, 1971; Boyes, 1975). Lignocaine metabolism has been the most extensively studied and the major metabolic pathway of this drug in humans (Fig 2) appears to be by oxidative N-dealkylation to monoethylglycinexylidide (MEGX). MEGX undergoes hydrolysis to form 2,6-xylidine which in turn is hydroxylated to form 4-hydroxy 2,6-xylidine. MEGX can also be further dealkylated to form

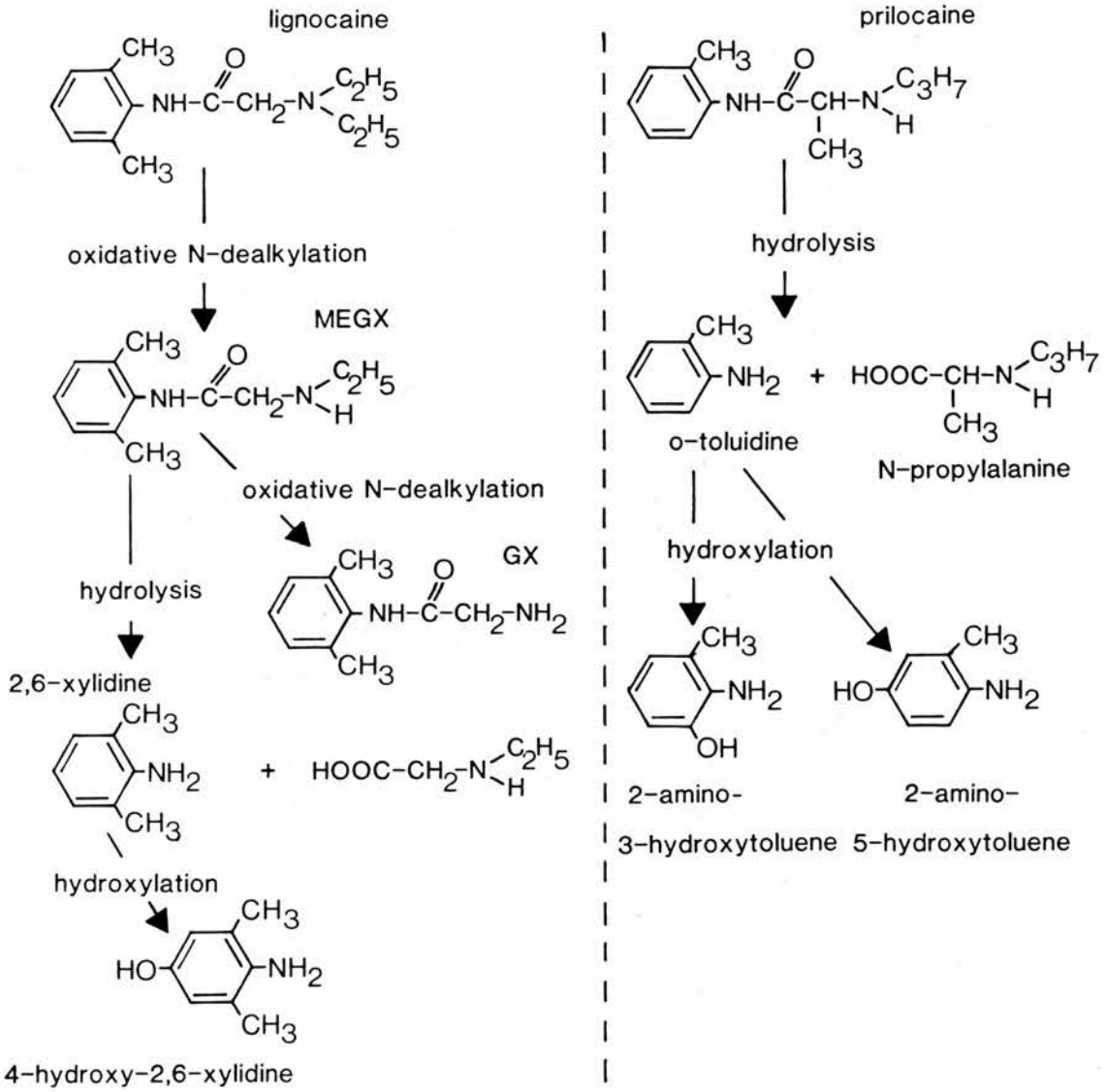
glycinexylidide (Keenaghan & Boyes, 1972). These metabolites are then excreted in the urine.

Metabolic studies of prilocaine have been less extensive. Geddes (1965) demonstrated that prilocaine could be metabolised by both liver and kidney slices from the rat and suggested that the initial metabolic step was hydrolysis of the amide linkage of the parent drug to form o-toluidine and N-propylalanine (Fig 2). Åkerman et al (1966a) confirmed these results with further in vitro work with tissue from a variety of animals. They reported that lung tissue, as well as liver and kidney tissue, were capable of metabolising prilocaine. Further work by Åkerman et al (1966b) identified two further metabolic products in the urine of rats and cats which were hydroxylation products of o-toluidine. These metabolites were identified as 2-amino-5-hydroxytoluene and 2-amino-3-hydroxytoluene (Fig 2) which have been implicated in the occurrence of methaemoglobinaemia after the administration of prilocaine. Subsequently Åkerman & Ross (1970) demonstrated that the enzyme activity in the liver responsible for prilocaine hydrolysis in vivo was located exclusively in the microsome fraction.

Because the toxic effects of the amide type local anaesthetics are mainly caused by their effect on the central nervous system, any factor reducing the amount and rate of these drugs reaching the central nervous system could assist in reducing toxic reactions to local

FIGURE 2

METABOLIC PATHWAYS OF LIGNOCAINE AND PRILOCAINE



anaesthetics. After intravenous injection or absorption from the site of administration, all the administered drug must pass through the lungs before reaching the brain. Englesson & Grevsten (1974) implicated the lungs as a major site of local anaesthetic distribution in the body. Tucker & Boas (1971) proposed that uptake of local anaesthetics by the lung could act as a buffer, by absorbing large quantities of these drugs, and thereby protecting the central nervous system by reducing high and possibly toxic blood concentrations. Also, tissue incubation experiments (Åkerman et al, 1966a) have demonstrated the possibility of metabolism of prilocaine in the lung.

The initial interest in non respiratory functions of lung tissue was stimulated by the work of Starling & Verney in 1925. They demonstrated rapid vasoconstriction in isolated perfused kidneys using defibrinated blood, but when the perfusate used was passed through a heart-lung preparation, this vasoconstrictor response was lost. The substance responsible for this action was later identified as 5-hydroxytryptamine (5-HT) and was shown to be inactivated by isolated perfused cat lungs (Gaddum et al, 1953).

Since then much work has been done on the ability of the lung to absorb and metabolise both endogenous and exogenous substances and several reviews have been published (Heinemann & Fishman, 1969; Brown, 1974;

Junod & de Haller, 1975; Junod, 1976; Löfström, 1978; Roth & Wiersma, 1979; Post, 1979).

Studies by Davis & Wang (1965) using simultaneous injections of indocyanine green and 5-HT showed a 33% removal of 5-HT by the pulmonary circulation in an intact dog preparation. However, much of the work on the uptake and metabolism of drugs by the lung has been performed with isolated perfused lung preparations. Alabaster & Bakhle (1970) found a 92% removal of ^{3}H-5-HT from perfusate by isolated perfused rat lungs and reported that most of the radioactivity recovered in the perfusate was probably 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5-HT. This process was found to be concentration independent in the concentration range 5-100 ng ml^{-1}. Other workers have found that removal of 5-HT from these perfusion systems to be mediated by a sodium dependent transport system (Junod, 1972b; Pickett et al, 1975; Iwasawa & Gillis, 1974) and that uptake of 5-HT is a saturable process. Monoamine oxidase has been shown to be responsible for 5-HT deamination by the rabbit lung (Gillis, 1973) but inhibition of this enzyme did not alter the ability of the lung to remove 5-HT from the perfusate.

Noradrenaline (NA) has also been shown to be accumulated by the lung in isolated perfused lung preparations (Alabaster & Bakhle, 1973; Nicholas et al, 1974; Iwasawa & Gillis, 1974) and, as for 5-HT, this process is saturable and sodium dependent. However, 5-HT is taken up to a greater extent than NA (Gillis et al,

1972; Gillis et al, 1974). The site of uptake of these two substances has been identified as the pulmonary capillary endothelial cells (Strum & Junod, 1972; Hughes et al, 1969).

Various factors have been shown to influence lung uptake of 5-HT and NA, such as cardiopulmonary bypass (Gillis et al, 1972; Gillis et al, 1974) after which extraction of both drugs from the circulation was increased. This was reported to be due to an increased surface area in the pulmonary vascular bed, across which these substances can be transported, caused by an increase in pulmonary artery pressure and possibly some stimulation of the active process accumulating 5-HT and NA. Anaphylaxis caused an increase in NA uptake by isolated guinea pig lung (Mathé & Volicer, 1977) which was attributed to improved membrane permeability. Conversely, competition with other drugs, eg imipramine, has been shown to reduce pulmonary uptake of both 5-HT and NA (Nicholas et al, 1974; Alabaster & Bakhle, 1970; Junod, 1972b).

Imipramine is a tricyclic antidepressant which inhibits the presynaptic uptake of NA and 5-HT. Furthermore, it has been proposed that inhibition of lung uptake of these substances by imipramine is due to direct competition for binding sites (Gillis, 1973). Conversely, Junod (1972a) found that 5-HT did not inhibit lung uptake of imipramine and suggested that this could be due to

imipramine binding to many more sites than 5-HT. Hence, it is easier to produce 5-HT uptake inhibition by imipramine than imipramine uptake by 5-HT. Junod also found that imipramine was not accumulated by the same sodium dependent mechanism operating for 5-HT and that the process was saturable.

Orton et al (1973) investigated lung uptake of a variety of basic and non-basic drugs by isolated perfused rabbit lungs and found that 50-95% of the basic drugs but only 10-20% of the non-basic drugs were accumulated by the lung tissue. Of the drugs investigated, only methadone was found to be metabolised by the lung to any degree. Other drugs which have been shown to be metabolised by intact lung preparations are propanolol (Kornhauser et al, 1980), ibuterol (Ryrfeldt & Nilsson, 1978) and mescaline (Roth et al, 1977). More comprehensive lists of drugs metabolised by the lung can be found in the reviews of Brown (1974), Post (1979) and Roth & Wiersma (1979). Comparison of results obtained using lung tissue or cell fraction incubations with results obtained from intact lung preparations, regarding drug metabolism by the lung, have shown wide discrepancies. Lung homogenates will metabolise imipramine and chlorcyclizine but intact perfused lung preparations have not confirmed these findings (Orton et al, 1973; Law et al, 1974; Eling et al, 1975). This is probably due to different cell types and enzymes coming into contact with these drugs which does not occur in the intact organ.

The uptake of lignocaine by lung tissue has been widely investigated. Katz (1968) found 3.2% of the total dose of lignocaine in the lungs of rats one minute after intravenous injection. Tucker & Boas (1971), in work on intravenous regional anaesthesia in humans, recognised the ability of the lung to absorb large quantities of local anaesthetics. Åkerman et al (1966a) found that after intraperitoneal, intramuscular and subcutaneous injections of a mixture of lignocaine and prilocaine in rats, the main difference in distribution to the tissues was in the lung, where prilocaine was concentrated to a much greater extent than lignocaine. Similar results were obtained after intraperitoneal injections of lignocaine and prilocaine in guinea pigs (Hansen et al, 1968 in Löfström, 1978).

Sjöstrand & Widman (1973) studied the effects of acidosis on the tissue distribution of bupivacaine in rabbits after a 20 min infusion of this drug. In normal rabbits they found a tissue : blood bupivacaine concentration ratio of 8:1, but in acidotic rabbits, much higher drug concentrations were attained by lung tissue, giving a ratio of 19.5:1. These workers stated that these changes could not be solely explained by an increase in organ blood flow combined with the increase in blood drug concentration. They suggested that with an increase in the water soluble form of bupivacaine (ie the cationic form) in acidotic animals, changes in the properties of the cellular membrane occurred together with an increase

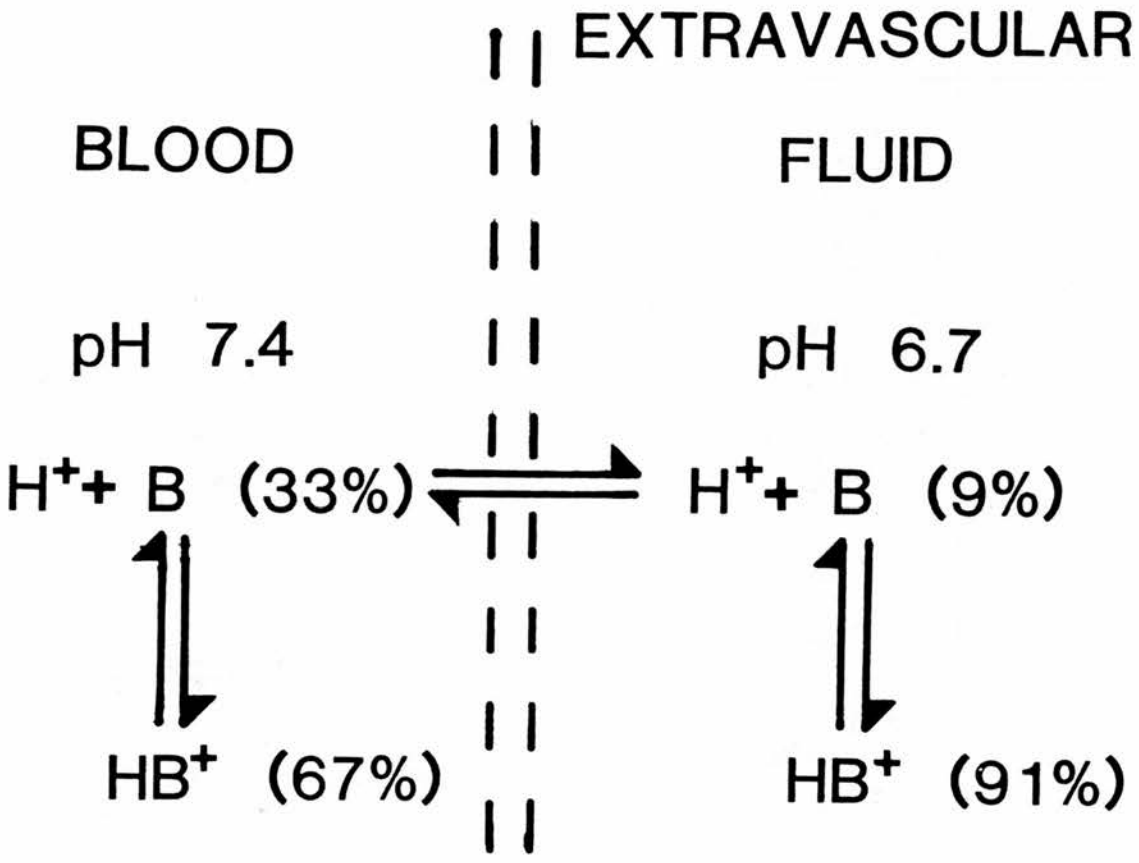
in tissue affinity for the drug and a combination of these factors resulted in the higher lung uptake of bupivacaine in acidosis.

Work on dogs has shown the arterial pH (7.4) to be greater than the pH of the pulmonary extravascular fluid (6.7)(Effros & Chinard, 1969) and this would suggest a mechanism for the accumulation of basic amines in lung tissue. As the uncharged base enters the extravascular tissue, it will equilibrate as uncharged base and cation, but there will be a greater proportion of cation in the extravascular fluid than in the blood (Fig 3). Hence a concentration gradient will be maintained between uncharged base in the blood and uncharged base in the tissue which will result in a greater concentration of drug in the pulmonary tissue than in the blood. Effros & Chinard also stated that after an infusion of 0.4 M sodium carbonate to produce alkalosis, tissue concentrations of cationic amines increased and that after an infusion of 0.3 M hydrochloric acid to produce acidosis, these concentrations decreased. This is the reverse situation to that found by Sjöstrand & Widman (1973).

In later work, Effros et al (1972) working with potentially 'anionic' amines (the barbiturates), found that the distribution of these compounds could not be fully explained by nonionic diffusion, as they also accumulated in lung tissue at higher concentrations than in blood. They concluded that the distribution of weak

FIGURE 3

A MECHANISM FOR THE ACCUMULATION OF A BASIC AMINE (pKa = 7.7) IN LUNG TISSUE. B = THE UNIONISED FORM OF THE COMPOUND AND HB^+ = THE IONISED FORM OF THE COMPOUND



acids and bases across cellular membranes was governed not only by the pKa of the substance, but also by the degree of lipid solubility of the compound and the extent of its protein binding. Åberg (1972) observed that the L-isomer of mepivacaine accumulated in lung tissue to a greater extent than its D-isomer. This stereo specific accumulation indicates that there may be specific binding sites and the affinity for these sites is dependent on the molecular configuration of the drug.

Post et al (1978) demonstrated that lignocaine was concentrated in rat lung slices by a non energy dependent process to a level seven times greater than the extracellular space and suggested that accumulation of lignocaine occurred not only within the cells, but also by being fixed to the cell surface by passive binding sites. Feinstein & Paimre (1969) suggested that this binding process would be by the attachment of the cationic form of lignocaine to negatively charged sites on the endothelium of the cells. Post also found that extraction of lignocaine by intact perfused lungs was biphasic in nature suggesting two processes of lignocaine accumulation. This biphasic nature of lung uptake has also been reported for methadone (Wilson et al, 1976), propranolol (Dollery & Junod, 1976) and imipramine (Anderson et al, 1974).

In further work with rat lung slices, Post et al (1979) demonstrated a greater tissue uptake at pH 8.0 of

lignocaine, bupivacaine and etidocaine than at pH 7.0 but could not relate the differences in uptake between these drugs to their lipid solubility. These workers found that lignocaine uptake by lung slices could be inhibited by nortriptyline and that this drug could also displace lignocaine from isolated perfused rat lungs. This observation was related to displacement of lignocaine from easily accessible binding sites on the capillary endothelium of the lung.

Using a technique involving the simultaneous intravenous injection of indocyanine green (an inert marker) and lignocaine in anaesthetised pigs (Bertler et al, 1978) it was demonstrated that 30-40% of lignocaine was absorbed in a single pass through the lungs. This supports the theory that the lung can act as a buffer to protect the central nervous system from high concentrations of local anaesthetic after intravenous administration. Bertler et al also showed that the efficiency of this uptake process decreased as the dose of lignocaine was increased. Using a similar technique with unanaesthetised human volunteers, Jorfeldt et al (1979) reported a 64% first pass pulmonary uptake of lignocaine. They proposed that, because the removal of lignocaine from the circulation was so rapid, binding of unionised lignocaine within the lipid part of the pulmonary endothelial cell membrane and extracellular binding of the cationic form of lignocaine to negatively charged sites on the pulmonary endothelial cell surface were the most likely processes involved in pulmonary

uptake of lignocaine.

It is apparent that the lungs are capable of absorbing large quantities of local anaesthetic and this is probably effective in reducing central nervous system toxicity of these drugs by reducing the blood concentrations reaching the brain. It has been proposed that the mechanisms involved in the uptake of the amide type local anaesthetics are intracellular absorption of the unionised form of the drug and binding of the cationic form of the drug to negatively charged sites on the pulmonary endothelial cell surfaces. Any factor altering the ability of the lung to accumulate these drugs could have an effect on the dose of local anaesthetic required to produce toxic symptoms. Such factors include changes in blood and tissue pH, changes in pulmonary arterial pressure, pulmonary disease and competition with other drugs.

The main aims of the studies reported in this thesis were to determine the pharmacokinetics of prilocaine in man and to assess the role of the lung in the distribution and metabolism of the amide type local anaesthetics.

S E C T I O N 1

PHARMACOKINETICS OF PRILOCAINE AND MEPIVACAINE IN HUMAN VOLUNTEERS

PHARMACOKINETICS OF PRILOCAINE AND MEPIVACAINE IN HUMAN VOLUNTEERS

INTRODUCTION

Of the amide type local anaesthetics, prilocaine is the only drug which has not been extensively investigated regarding its pharmacokinetic characteristics. Lignocaine pharmacokinetics in man have been most widely investigated (Boyes et al, 1971; Rowland et al, 1971; Benowitz et al, 1974) and recently reviewed (Benowitz & Meister, 1978). The pharmacokinetics of bupivacaine and mepivacaine were determined in man by Reynolds (1971) and a comparison of bupivacaine with etidocaine has been undertaken (Scott et al, 1973). Lignocaine, mepivacaine, etidocaine and bupivacaine were studied by Tucker & Mather (1975) and the results of other workers reviewed. Further reviews have been published by these authors (Mather & Tucker, 1978; Tucker & Mather, 1979). Eriksson (1966) and Lund & Covino (1967) have presented data which suggested a much greater total body clearance of prilocaine as compared to lignocaine.

This study was designed to determine the pharmacokinetic variables of prilocaine and mepivacaine in healthy male volunteers, using the results obtained for mepivacaine as a comparison with previously published data.

MATERIALS AND METHODS

Ten healthy male volunteers (Table 1-1) agreed to take part in the study, having been fully informed of the nature of the drugs and the experimental protocol. Five volunteers received both an infusion of 250 mg prilocaine hydrochloride and an infusion of 250 mg mepivacaine hydrochloride on two separate occasions separated by at least seven days. The other five volunteers received only the 250 mg prilocaine hydrochloride infusion.

Prilocaine hydrochloride (1% Citanest, Astra Chemicals Ltd, Watford, GB) or mepivacaine hydrochloride (2% Carbocaine, Pharmaceutical Manufacturing Co, Bolton, GB) were diluted with saline to give a 5 mg ml^{-1} solution. Each infusion was given over a 12.5 min period into a vein in the dorsum of the right hand at a rate of 4 ml min^{-1} (20 mg min^{-1}) through an indwelling needle (Butterfly, 23-G, Abbot Laboratories Ltd, Kent, GB) using a Harvard syringe infusion pump (Harvard Apparatus, Millis, Mass, USA).

Venous blood samples (5 ml) were withdrawn during and after the infusion through an indwelling cannula (Venflon 18-G, 45 mm long, Viggo AB, Helsinborg, Sweden) and transferred to heparinised tubes (Brunswick, 10 ml lithium heparin tubes, Sherwood Medical Ltd, Co Antrim, N Ireland). The samples were centrifuged, the plasma separated, transferred to plain plasma tubes (Seward Laboratory, East Grinstead, West Sussex, GB) and stored

TABLE 1-1

HUMAN VOLUNTEER DATA

Volunteer	Age (yr)	Height (cm)	Weight (kg)	Smoker
1 (HB)	33	183	76	No
2 (GD)	32	188	77	No
3 (DF)	28	166	60	No
4 (AN)	31	179	64	No
5 (DL)	32	182	82	Yes
6 (RA)	27	179	72	Yes
7 (JM)	30	175	67	No
8 (RM)	33	178	60	No
9 (GO)	23	171	67	No
10 (BM)	29	175	71	Yes
$\bar{x}$	30	178	70	
SD	3	6	7	

in a freezer until analysis.

The volunteers who received infusions of prilocaine hydrochloride and mepivacaine hydrochloride had blood samples withdrawn at regular intervals for four hours. Those subjects receiving only the prilocaine infusion had blood samples withdrawn for eight hours. The electrocardiogram (ECG) was monitored on all occasions for the first 60 minutes of the experiment and systemic arterial pressure was monitored by a semi automatic sphygmomanometer (Parama UM-15LN, Parama Co Ltd, (1947) Co, Blackpool, GB). Plasma drug concentrations were determined by a gas liquid chromatographic technique as described in Appendix 1. The concentrations of both compounds have been expressed in terms of the drug hydrochloride and throughout this work, unless otherwise stated, prilocaine hydrochloride is referred to as prilocaine, mepivacaine hydrochloride as mepivacaine and lignocaine hydrochloride monohydrate as lignocaine.

Pharmacokinetic analysis of the plasma drug concentration data was based on a two compartment open model, details of which are given below.

Pharmacokinetic Analysis Based on a Two Compartment Open Model

The division of the body into two compartments to describe the fate of an administered drug is not based on anatomical considerations. The division is determined by a mathematical description of the shape of the post-

absorption time/concentration profile as determined by analysis of blood samples taken at various times after the administration of the drug. The shape of the time/drug concentration profile on a semilogarithmic plot (Fig 1-1) has two distinct phases. These can generally be thought of as an initial distribution phase during which the drug blood concentration drops rapidly as it is distributed to, and absorbed by, the different tissues of the body, followed by the elimination phase which reflects the loss of drug from the body either by metabolism or excretion of unchanged drug.

The mathematical derivations used to describe the two compartment model here are basically as previously presented by Gibaldi & Perrier (1975).

The graph in Fig 1-1 represents the result of a single instantaneous intravenous bolus injection into the system. The resolution of the curve into its two linear phases gives two intercept points, A and B, on the concentration axis and these intercept values can be related to the blood drug concentration at any time after the injection of the drug:

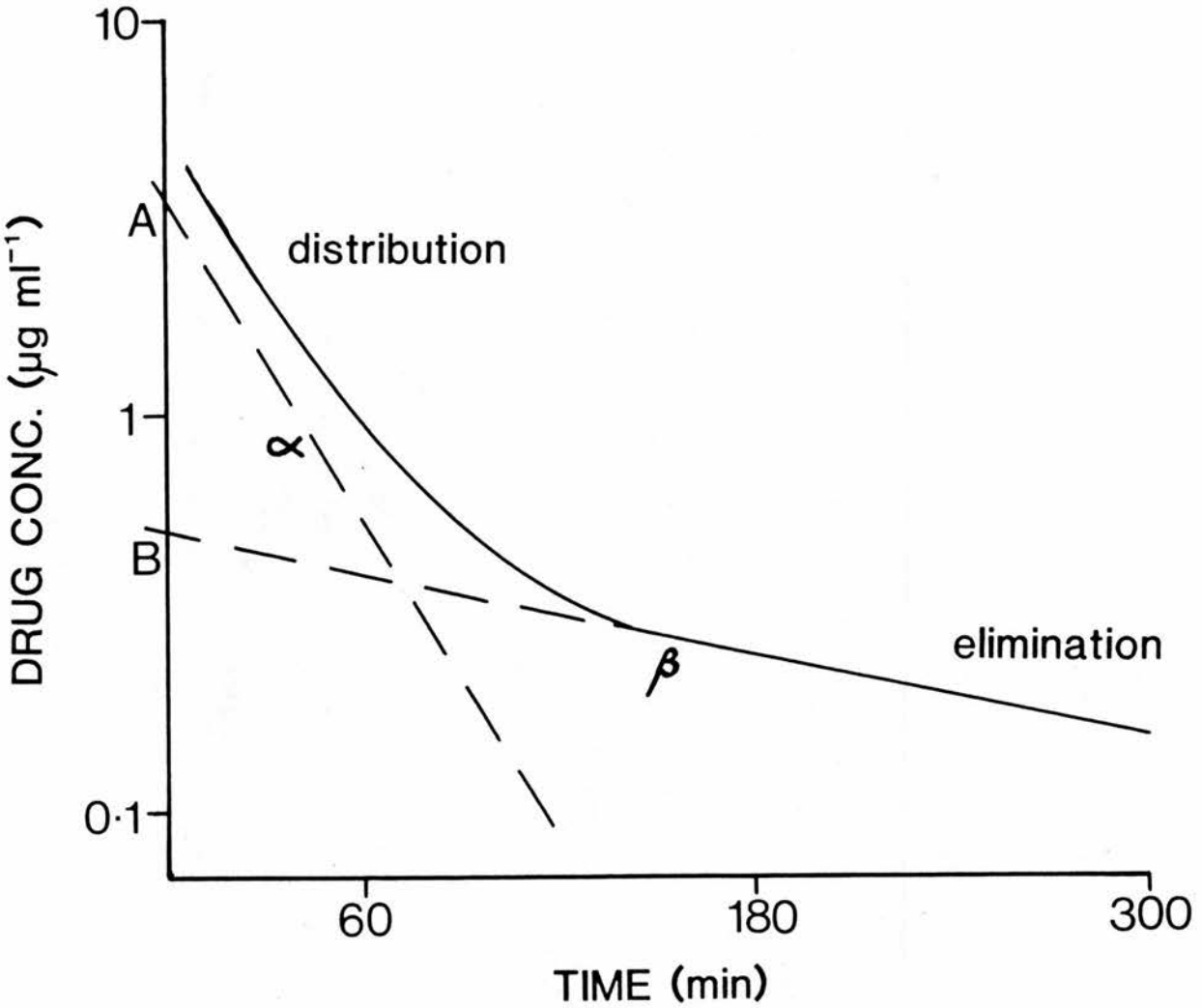
$$C_t = Ae^{-\alpha t} + Be^{-\beta t} \qquad 1$$

Where C_t = blood drug concentration ($\mu g\ ml^{-1}$) at time (t) after drug injection

α = the slope of the rapid distribution phase of the drug (min^{-1})

FIGURE 1-1

STYLISED SEMILOGARITHMIC PLOT OF DRUG CONCENTRATION .v. TIME AFTER A SINGLE INTRAVENOUS BOLUS INJECTION OF DRUG



β = the slope of the slower elimination phase of the drug (min^{-1})

t = the time (min) after drug injection

A and B = the intercept values ($\mu g\ ml^{-1}$)

The half lives of both the α and β phases represent the time taken for the blood drug concentration to be halved. These are determined:

$$t_{\frac{1}{2}\alpha}(min) = \frac{0.693}{\alpha} \qquad 2$$

$$t_{\frac{1}{2}\beta}(min) = \frac{0.693}{\beta} \qquad 3$$

Fig 1-2 is a schematic representation of a two compartment system. The central compartment equilibrates rapidly and anatomically it is probably represented by the blood and the highly perfused body tissues such as the heart, lungs, liver, brain and kidneys. The peripheral compartment equilibrates slowly and is probably formed by the less perfused tissues such as muscle and body fat. The k values are rate constants:

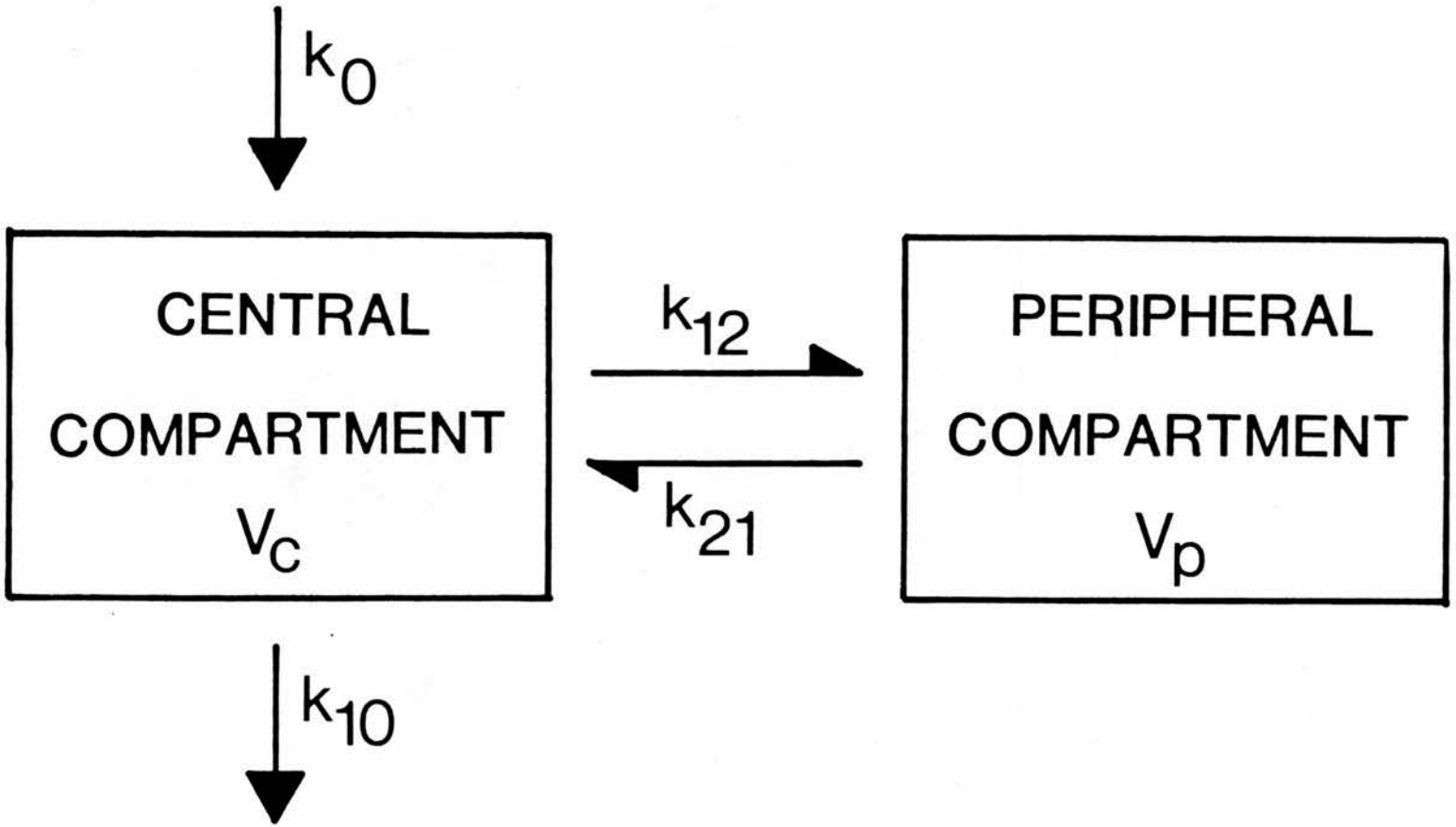
k_O = input rate

k_{10} = elimination rate constant

k_{12} and k_{21} = rate constants of transfer between the two compartments

FIGURE 1-2

SCHEMATIC REPRESENTATION OF A TWO COMPARTMENT OPEN MODEL



The rate constants are derived from A, B, α and β:

$$k_{21} = \frac{A\beta + B\alpha}{A + B} \quad 4$$

$$k_{10} = \frac{\alpha\beta}{k_{21}} \quad 5$$

As $\alpha + \beta = k_{12} + k_{21} + k_{10}$ then

$$k_{12} = \alpha + \beta - k_{21} - k_{10} \quad 6$$

The area under the plasma (or blood) concentration curve can be both measured (AUC_M), using the trapezoidal rule with correction for infinite time, or calculated (AUC_C):

$$AUC_C = \frac{A}{\alpha} + \frac{B}{\beta} \quad 7$$

A comparison of AUC_C and AUC_M gives some indication as to the validity of the calculated variables, ie if AUC_C and AUC_M are very similar then it is probable that the calculated pharmacokinetic variables are accurate.

The V_c and V_p values are hypothetical volumes (expressed as litres) of each compartment which effectively relate to the volume of blood required to account for the amount of drug in each compartment.

The volume of the central compartment (V_c) is calculated:

$$V_c(1) = \frac{X_o}{A + B} \quad 8$$

Where X_o = the initial dose of the drug (mg)
and A and B are as previously defined

To determine V_p it is necessary to determine the total volume of distribution of the body (V_B) which is calculated:

$$V_B = \frac{X_o}{(\frac{A}{\alpha} + \frac{B}{\beta})\beta} \quad 9$$

which is the same as:

$$V_B = \frac{X_o}{AUC_C . \beta} \quad 10$$

$$\text{hence } V_p = V_B - V_c \quad 11$$

The pharmacokinetic variable used to relate drug concentration to the rate of drug elimination is the clearance value (Cl). This relates to the volume of blood from which all drug is apparently removed in a certain unit of time and is defined:

$$Cl\ (l\ min^{-1}) = \frac{X_o}{AUC} \quad 12$$

Throughout the results presented in this work, Cl_M represents the value obtained where AUC_M has been used in equation 12 and Cl_C the value obtained where AUC_C has been used in equation 12.

In Section 4 of this work the term Cl_{ss} has been used. This term represents the clearance value obtained from drug concentration data obtained after steady state concentrations of drug have been reached following an intravenous infusion. Cl_{ss} is defined:

$$Cl_{ss} \ (l \ min^{-1}) = \frac{k_o}{C_{ss}} \qquad 13$$

where C_{ss} = drug concentration at steady state ($\mu g \ ml^{-1}$)

k_o = rate of infusion ($mg \ min^{-1}$)

As mentioned previously, these derivations have been based on an instantaneous bolus injection of drug. In the human volunteer study reported here, prilocaine and mepivacaine were infused intravenously over a period of 12.5 minutes and in other experiments reported later, drug has been infused intravenously over longer time periods. As a result of this the determined A and B intercept values from these experiments would not be the same had the total dose been given as an instantaneous intravenous bolus injection. These intercept values obtained after an intravenous infusion have been termed A' and B' and have been corrected for infusion time using equations derived by Loo & Riegelman (1970).

$$A = \frac{\beta\tau}{1 - e^{-\alpha\tau}} \cdot A' \qquad 14$$

$$B = \frac{\beta\tau}{1 - e^{-\beta\tau}} \cdot B' \qquad 15$$

where τ = the infusion time (min)

and the other terms are as previously defined

RESULTS AND DISCUSSION

Heart Rate, Blood Pressure and ECG

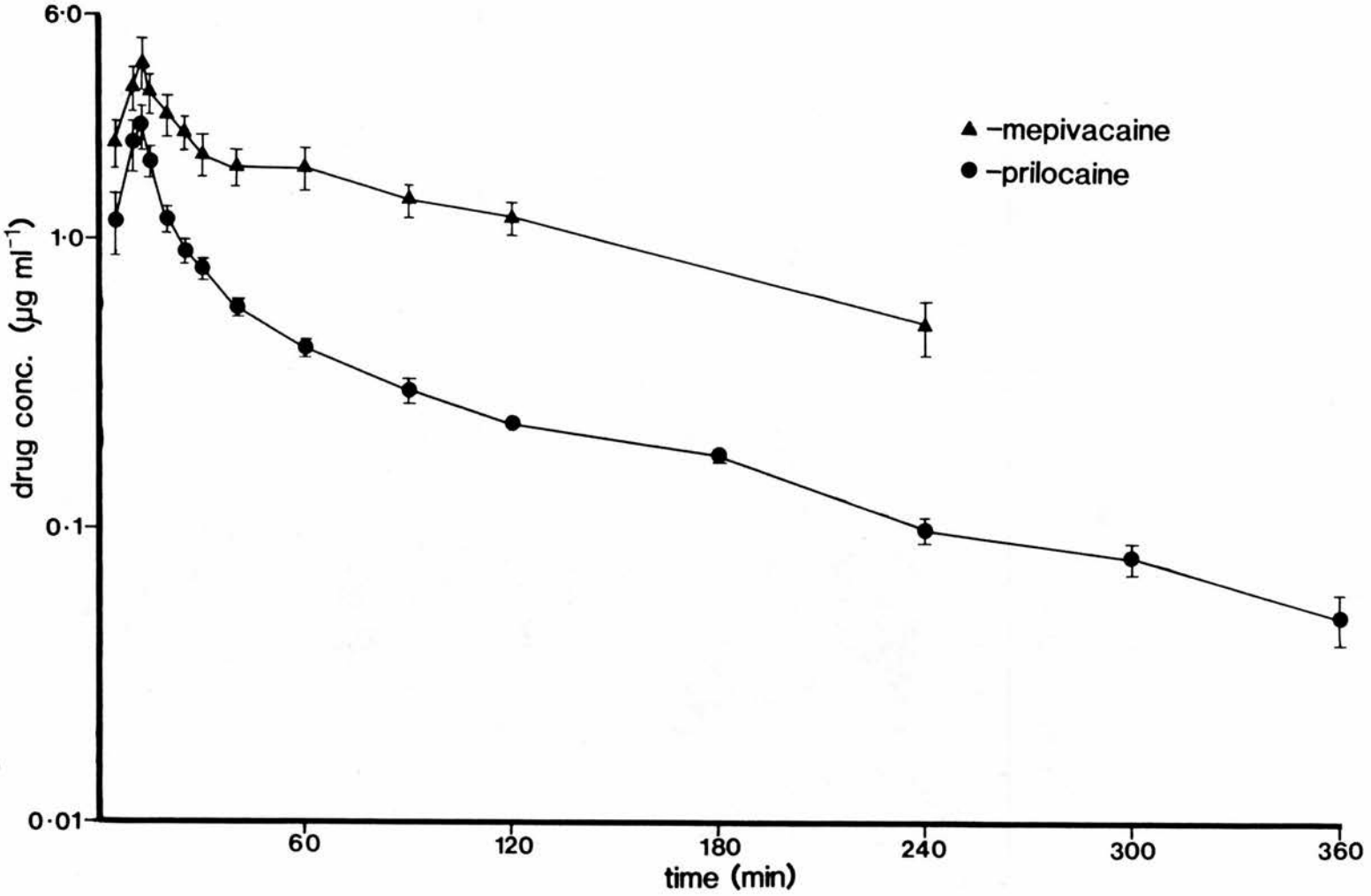
There were no significant alterations in heart rate, blood pressure (Appendix 5) or ECG throughout the monitoring period.

Prilocaine and Mepivacaine Plasma Concentrations Appendices 6 and 7

The time/concentration profiles for both drugs are illustrated in Fig 1-3. As peak plasma concentrations of the drugs were reached towards the end of the infusion, one subject receiving prilocaine and two receiving mepivacaine reported mild toxic symptoms (numbness of the lips and tongue and feeling light headed). After terminating the infusion, plasma concentrations of prilocaine fell very rapidly at first then a slower decline in concentrations occurred until, at 420 minutes after the start of the infusion, the plasma concentrations of prilocaine were less than the minimum detectable limit of the assay (ie less than 0.05 $\mu g\ ml^{-1}$).

FIGURE 1-3

SEMILOGARITHMIC PLOT OF PRILOCAINE AND MEPIVACAINE PLASMA CONCENTRATIONS IN HUMAN VOLUNTEERS AFTER INTRAVENOUS INFUSION (mean values ± SE bars)



Pharmacokinetic Analysis

Data derived from the pharmacokinetic analysis of plasma prilocaine concentrations are shown in Table 1-2. The calculated and measured area under curve values were very similar indicating that the calculated data represented an accurate description of the blood level results. The half life of elimination of prilocaine was 97 minutes which is similar to the value reported for lignocaine (96 minutes) by Tucker & Mather (1975). The total body clearance for prilocaine was calculated to be 2.42 l min^{-1} with only two subjects having values less than 2 l min^{-1}. It is generally accepted that amide type local anaesthetics are metabolised almost entirely by the liver and that only small amounts of the drugs are excreted unchanged in the urine (Boyes, 1967; Eriksson & Granberg, 1965; Åkerman et al, 1966a). Therefore, clearance values for these drugs must be less than liver blood flow, which has been reported as ranging from 1.5 l min^{-1} (Tucker et al, 1977; Ganong, 1975) to 1.7 l min^{-1} (Price et al, 1960). The clearance value of prilocaine exceeds the highest accepted level of liver blood flow suggesting that prilocaine is subject to some extra-hepatic metabolism.

Tucker et al (1977) reported that prolonged infusions (150 min) of lignocaine, bupivacaine and etidocaine increased liver blood flow, with a maximum value of 2.0 l min^{-1} obtained for the lignocaine infusion.

TABLE 1-2

PHARMACOKINETIC VARIABLES OF PRILOCAINE IN HUMAN VOLUNTEERS

(mean values ± SD)

	$\bar{x}$	SD
α (min^{-1})	0.101	0.033
β (min^{-1})	0.0079	0.0029
$t_{½\alpha}$ (min)	7.5	2.2
$t_{½\beta}$ (min)	97	30
A ($\mu g\ ml^{-1}$)	3.02	1.99
B ($\mu g\ ml^{-1}$)	0.61	0.22
AUC_C ($\mu g\ ml^{-1}.min$)	108	22
AUC_M ($\mu g\ ml^{-1}.min$)	109	26
Cl ($l\ min^{-1}$)	2.42	0.62
V_B (l)	343	130
V_c (l)	87	40
V_p (l)	256	105
k_{10} (min^{-1})	0.035	0.019
k_{21} (min^{-1})	0.024	0.006
k_{12} (min^{-1})	0.062	0.046

The data for each volunteer can be found in Appendix 8

It is possible that the short prilocaine infusion given in this study (12.5 min) increased the hepatic blood flow of the volunteers somewhat but probably not enough to account for the high prilocaine clearance values. The data of Tucker et al (1977) showed that during the first 30 minutes of the infusion of lignocaine, bupivacaine and etidocaine there was very little change in hepatic blood flow. Also, Lalka et al (1976) using a shorter infusion of lignocaine (8 min) reported no change in the indocyanine green clearance (a good index of liver blood flow as this dye is entirely eliminated by the liver) during the experiment. The mean value for the volume of distribution of prilocaine was relatively large (343 l), this being mainly due to the large volume of distribution of the peripheral compartment (256 l).

The volunteer who experienced toxic symptoms during the prilocaine infusion (GO) had the lowest clearance value (1.61 l min^{-1}) of all ten subjects and also had the highest peak plasma concentration (5.27 $\mu g\ ml^{-1}$). With this same subject plasma prilocaine concentrations decreased very rapidly after the end of the infusion and the $t_{\frac{1}{2}\beta}$ of 64 minutes was the second fastest obtained in this group of volunteers. The volume of distribution in this subject was relatively small (148 l) compared to the mean value of 343 l.

Table 1-3 gives some of the pharmacokinetic data for prilocaine and mepivacaine from this work and

TABLE 1-3

COMPARISON OF PHARMACOKINETIC VARIABLES OF VARIOUS LOCAL ANAESTHETICS

			from Tucker & Mather (1975)			
	Prilocaine	Mepivacaine	Mepivacaine	Lignocaine	Bupivacaine	Etidocaine
$t_{\frac{1}{2}\beta}$ (min)	97	125	114	96	210	156
Cl (l min^{-1})	2.42	0.68	0.78	0.95	0.47	1.22
V_B (l)	343	115	150	212	209	666

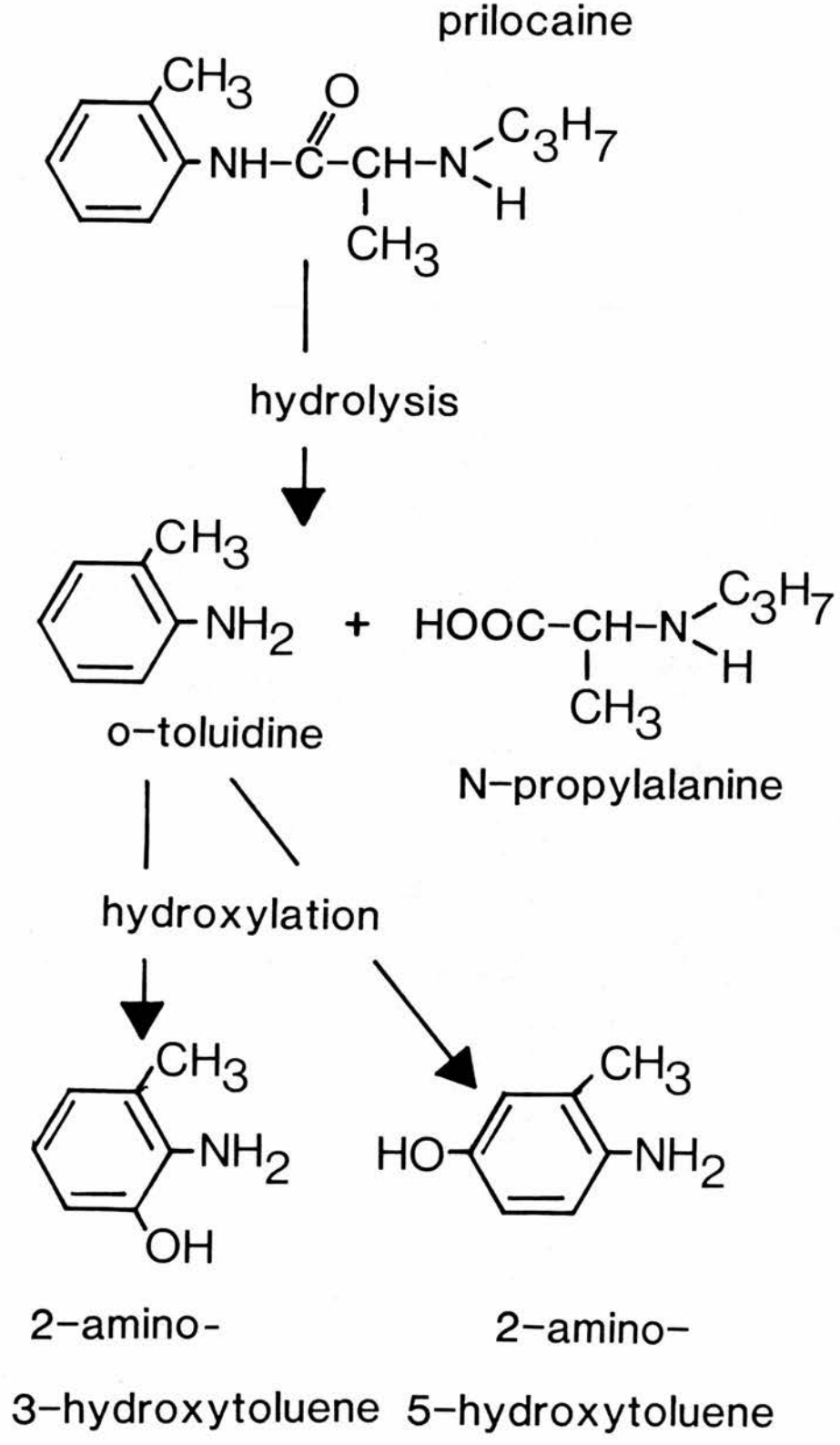
The complete results for the pharmacokinetic analysis of the mepivacaine concentration data can be found in Appendix 9.

provides a comparison with pharmacokinetic data for mepivacaine and other local anaesthetics presented by Tucker & Mather (1975). It is apparent that the two sets of mepivacaine results are similar, implying that the high clearance value obtained for prilocaine in this study was not due to the volunteers having unusually high liver blood flows. The clearance value of prilocaine is twice that of etidocaine, the only other of these drugs with a clearance value in excess of 1 l min^{-1}. Although prilocaine has a low lipid solubility (Covino & Vassallo, 1976) it has a relatively large volume of distribution. The only amide type local anaesthetic with a larger volume of distribution is etidocaine and it is over 100 times more lipid soluble than prilocaine.

SKF 525A, an inhibitor of oxidative metabolism, was found to inhibit lignocaine but not prilocaine metabolism (Åkerman et al, 1966a) indicating that prilocaine, unlike lignocaine, does not undergo oxidative degradation as a first step in its metabolism. Geddes (1965) and Åkerman et al (1966a) suggested that the primary metabolic pathway of prilocaine was by hydrolysis of the amide bond, forming o-toluidine and N-propylalanine with further hydroxylation products identified by Åkerman et al (1966b) (Fig 1-4). The amidase enzyme responsible was believed to be present not only in the liver, but also in the lung and kidney. In later work, Åkerman & Ross (1970) reported that the enzyme activity in the liver, responsible for the hydrolysis of prilocaine,

FIGURE 1-4

METABOLIC PATHWAY OF PRILOCAINE



was located in the microsome fraction and that this system had the same pH dependency as reported for the hydrolysis of the lignocaine metabolite monoethylglycinexylidide (Hollunger, 1960b) and suggested that the two fractions were identical. Work by Åkerman et al (1966a) using slices and homogenates of lung and kidney from rabbits and cats demonstrated that both these tissues were capable of metabolising prilocaine to a similar degree. Geddes (1965) also reported that kidney slices from rats metabolised prilocaine. However, the calculated minimum extra-hepatic clearance of over 700 ml min^{-1} observed in this study represents 50-60% of the renal blood flow but only 10-15% of pulmonary blood flow, hence the lungs seemed to be the more attractive organ to propose as a site of extra-hepatic metabolism.

The following experiments were designed in an attempt to identify the extra-hepatic site of prilocaine metabolism and elucidate the contribution of the lungs to the distribution of local anaesthetics in the body.

S E C T I O N 2

LUNG UPTAKE OF PRILOCAINE AND LIGNOCAINE BY RAT LUNG PREPARATIONS

ISOLATED PERFUSED RAT LUNG PREPARATIONS

INTRODUCTION

In vivo studies on the tissue distribution of lignocaine and prilocaine in rats (Åkerman et al, 1966a) showed that prilocaine was found to be accumulated to a much greater extent in the lung than lignocaine.

Post et al (1978) found the uptake of lignocaine by rat lung slices to be dependent on the pH of the incubation medium and Englesson & Grevsten (1974) recognised the effect blood pH could have on the uptake and distribution of local anaesthetics.

The following experiments were designed to quantify the lung uptake of lignocaine and prilocaine by an isolated perfused rat lung preparation and to observe any changes in this uptake at perfusate pH values at the extremes of physiological tolerance.

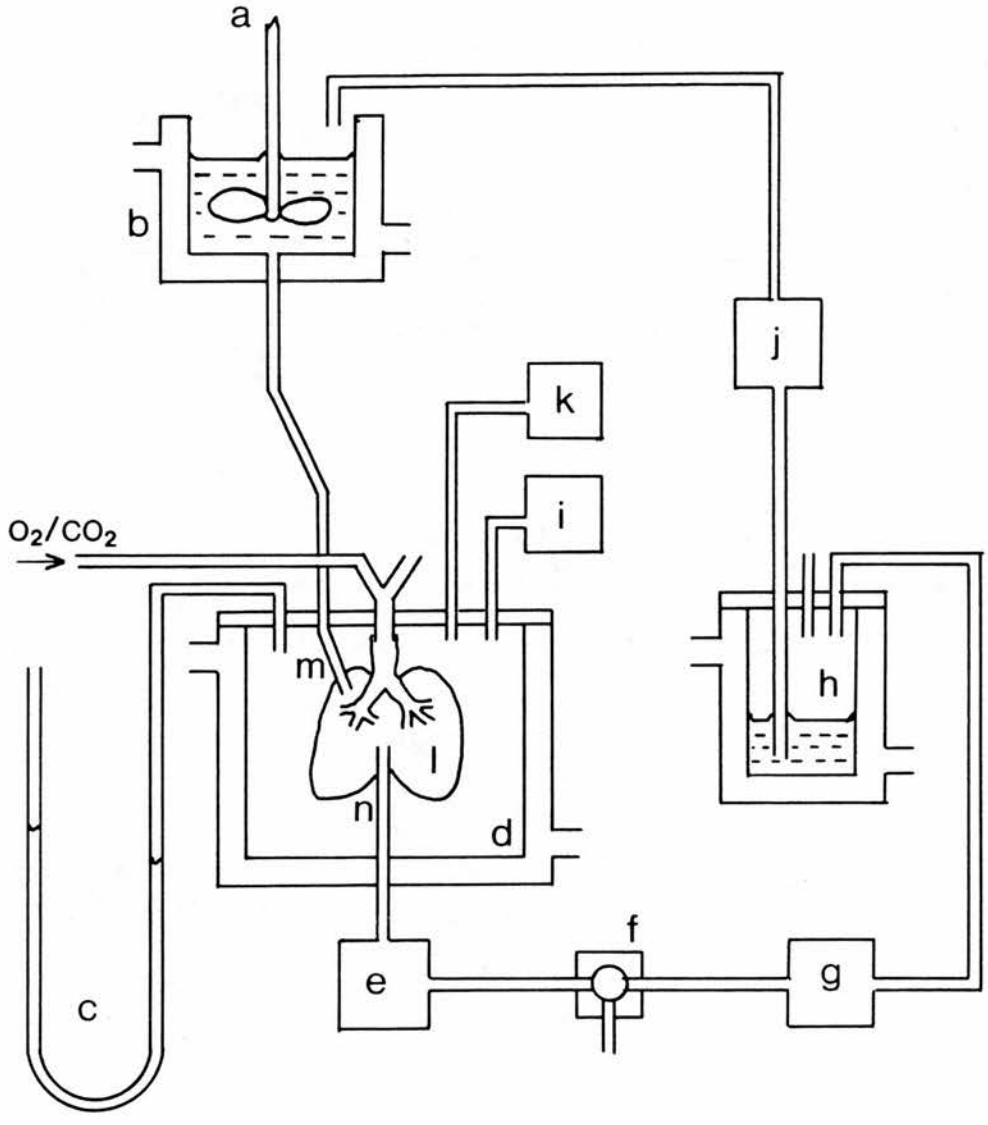
MATERIALS AND METHODS

The lung preparation and perfusion system was basically that described by Ryrfeldt & Nilsson (1978). Male Sprague-Dawley rats weighing between 250 and 300 g were used in these experiments. Heparin (500 IU) was injected into a tail vein of the rat, then the animal was anaesthetised with pentobarbitone sodium (20-40 mg) given intraperitoneally. A tracheotomy was performed

and then the thorax opened by a midline incision to reveal the heart and lungs. The circulatory system was drained through an incision in the right ventricle and through this incision the pulmonary artery was catheterised using a short length (approximately 5 cm) of silastic tubing, care being taken to avoid the introduction of air bubbles into the system. An incision was then made in the left ventricle and the pulmonary venous return catheterised with a similar length of silastic tubing via the left ventricle and left atrium. Both catheters were firmly sutured to prevent leakage of perfusate from the system. The lung and heart were then cut free from the animal and suspended in an artificial thoracic chamber. The pulmonary artery catheter was connected to the perfusate reservoir and the pulmonary venous catheter connected to the outflow port of the chamber (Fig 2-1). The lungs were ventilated with warmed humidified carbogen (95% O_2/5% CO_2) using alternating negative pressure (-5 to -50 mm water) created by an animal respirator (Harvard Rodent Respirator, Model 681, Harvard Apparatus, Millis, Mass, USA). The lungs were perfused with Kreb's-Ringer bicarbonate buffer (Appendix 10) containing 4.5% bovine albumin (Fraction V, Sigma, Poole, Dorset, GB) and 0.1% glucose. The perfusate was maintained within the pH range 7.35 to 7.40 for most experiments, countering the tendency of the pH to rise by intermittently blowing carbon dioxide into the perfusate reservoir. The pH of the perfusion medium was changed to the ranges 7.00 to

FIGURE 2-1

DIAGRAMMATIC REPRESENTATION OF THE ISOLATED PERFUSED RAT LUNG SYSTEM



a. stirrer
b. perfusate reservoir
c. pressure manometer
d. thoracic chamber
e. electromagnetic flowmeter
f. sampling port
g. pH/pO_2 electrodes
h. perfusate reservoir
i. vacuum pump
j. peristaltic pump
k. respirator
l. lung
m. pulmonary arterial catheter
n. pulmonary venous catheter

7.05 and 7.80 to 7.85 for some experiments; lowering the pH by bubbling carbon dioxide through the perfusate and increasing the pH by the addition of small amounts of 0.1 molar sodium hydroxide solution. The pH and pO_2 were constantly monitored using pH and pO_2 electrodes in a flow cell (Radiometer, Copenhagen) built into the perfusion system. The system was maintained at a temperature of 37°C by a system of circulating warmed water jackets. The perfusate flow rate was monitored using an electromagnetic flowmeter and was adjusted to 15 ml min^{-1} by raising or lowering the height of the perfusate reservoir. Furthermore, control experiments were also run with no lungs in the system, using the same perfusion conditions as listed above, to determine to what extent the drugs were adsorbed by the tubing in the system.

After the lung had equilibrated in the system, ie after the perfusate flow, pH and pO_2 had stabilised, a 250 µl bolus injection of the tritiated drug was introduced into the system through a rubber union between the perfusate reservoir and the pulmonary artery catheter. Samples of perfusate were then withdrawn from a sampling port in the system at the following times after drug injection: 0, 1, 1.5, 2, 2.5, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 25, 30 minutes. The recirculating perfusion system contained an initial volume of approximately 62 ml (including the 250 µl injection and the dead space of the catheters and lungs). ^{3}H-lignocaine hydrochloride (specific activity 37.5 µCi mg^{-1}) and ^{3}H-prilocaine

hydrochloride (specific activity 29.12 μCi mg^{-1})(Astra Lakemedel AB, Sodertalje, Sweden) were dissolved in distilled water at concentrations of 650 μg ml^{-1} and 657 μg ml^{-1} respectively. Thus a 250 μl injection would produce perfusate lignocaine and prilocaine concentrations of approximately 2.6 and 2.7 μg ml^{-1} respectively, assuming that uniform mixing and no absorption of the drugs were to occur.

To a 100 μl sample of perfusate 10 ml of a scintillation cocktail (Instagel, Packard, HP Svierge AB, Lund, Sweden) was added and sample radioactivity was assayed using a liquid scintillation spectrometer (Packard Tri-Carb 2425). Each sample was counted three times for a period of four minutes and the mean disintegrations per minute (dpm) calculated by a computer programmed to automatically compensate for quenching effect.

Lung tissue was homogenised in distilled water and weighed samples oxidised using a Packard Tri-Carb Sample Oxidiser (Model 306). The radioactive residue was mixed with 15 ml of a scintillation cocktail (Monophase, Packard) and each sample counted three times for a period of four minutes then the mean dpm calculated as previously described. The efficiency of the oxidation system was determined by running lung homogenate samples of known activity through the same procedure as for the unknown samples and calculating the percent recovery of this activity.

RESULTS AND DISCUSSION

The amount of drug in each sample has been expressed as a percentage of the expected drug concentration in a theoretical perfusate sample taken from the system at equilibrium with no absorption of the drug occurring. These values were calculated from the relationship:

$$\% \text{ drug in sample} = \frac{\text{sample dpm}}{\text{theoretical dpm}} \times 100$$

It should be noted that if any metabolism of either drug occurred in this preparation then radioactivity associated with the metabolites would be included in these results.

Post et al (1978), using a similar rat lung perfusion system, found that ^{14}C-lignocaine disappeared from the perfusion medium when the lung was excluded from the system. Control experiments indicated that the mean drug loss from the perfusate with no lung in the system was 11% for both lignocaine and prilocaine and the data quoted for lung uptake of these drugs has been appropriately corrected.

With both the lignocaine and prilocaine experiments, an equilibrium was attained in the system by five minutes (Fig 2-2). With lignocaine in the system, at physiologic pH (range 7.35 to 7.40) a mean amount of 9% (using data from 5-30 min) of the drug was removed from the perfusate and for prilocaine this was 18% (Table 2-1). The difference between the amounts of lignocaine and prilocaine remaining in the system at equilibrium was

FIGURE 2-2

MEAN % OF THE DOSE OF PRILOCAINE AND LIGNOCAINE REMAINING IN RAT LUNG PERFUSATE AT TIMES UP TO 30 min AFTER DRUG INJECTION INTO THE ISOLATED PERFUSED RAT LUNG SYSTEM. THE PLOTTED VALUES HAVE BEEN CORRECTED FOR CONTROL EXPRIMENTAL DATA. (SE bars are too small to be indicated) ▲ = PRILOCAINE; ● = LIGNOCAINE

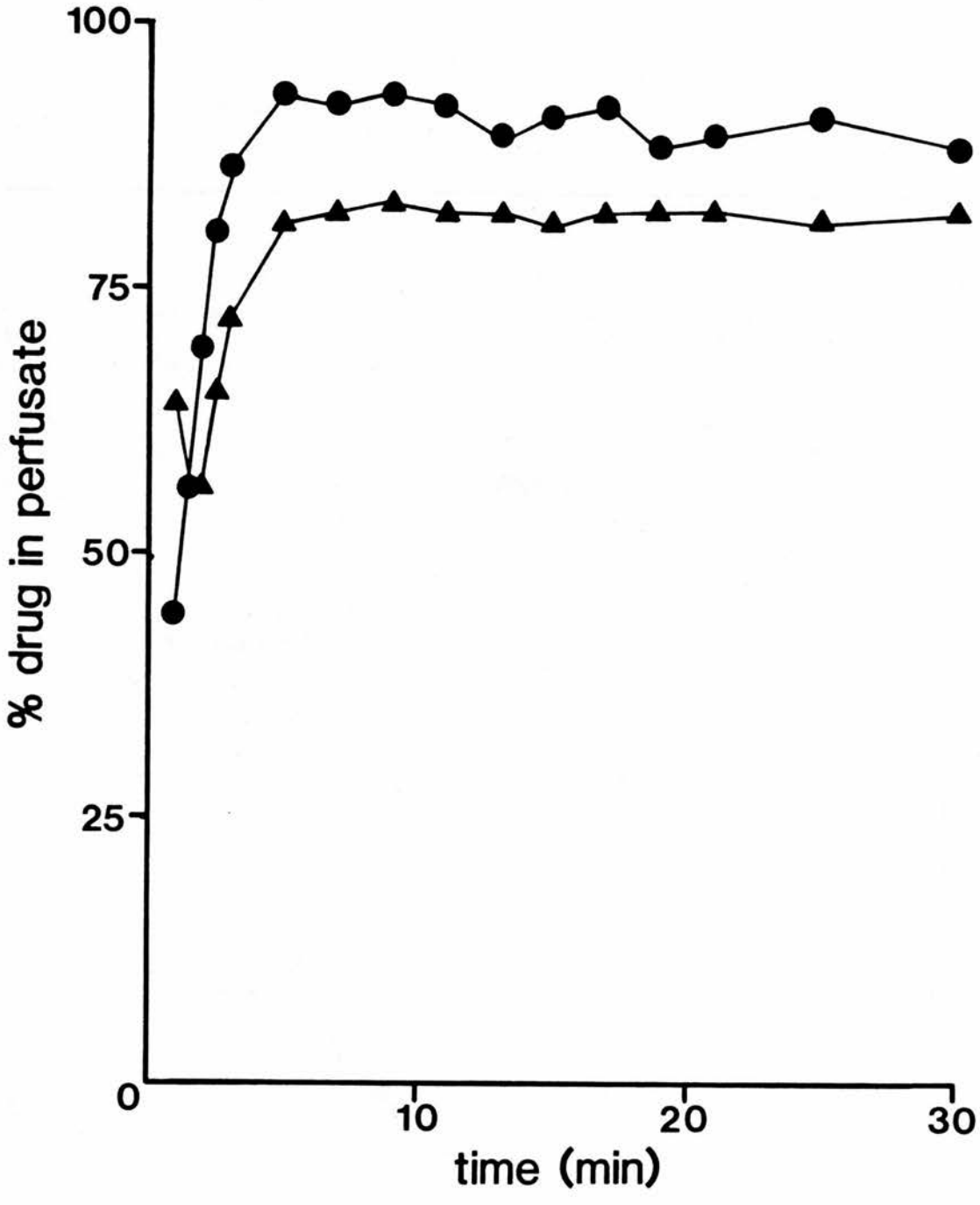


TABLE 2-1

AMOUNT OF DRUG IN PERFUSATE EXPRESSED AS A % OF EXPECTED CONCENTRATIONS AT EQUILIBRIUM. THE DATA HAS BEEN CORRECTED FOR DRUG LOSS FROM THE PERFUSATE IN CONTROL EXPERIMENTS

TIME (min)		5	7	9	11	13	15	17	19	21	25	30
Lignocaine pH 7.35-7.40 (6 preparations)	$\bar{x}$	93	92	93	92	89	91	92	88	89	91	88
	SE	2	2	1	1	2	1	1	2	2	1	1
Prilocaine pH 7.35-7.40 (6 preparations)	$\bar{x}$	81	82	83	82	82	81	82	82	82	81	82
	SE	1	1	1	1	1	1	1	1	1	1	1
Lignocaine pH 7.00-7.05 (2 preparations)	$\bar{x}$	90	91	93	93	92	92	92	92	92	92	92
Lignocaine pH 7.80-7.85 (2 preparations)	$\bar{x}$	90	94	94	93	94	93	93	92	92	92	91

Using Student's t test for two means, the results for lignocaine in the pH range 7.35-7.40 (5-30 minutes) were significantly greater than those for prilocaine ($p < 0.001$)

statistically significant ($p < 0.001$).

This difference was reflected by the results of the analysis of lung tissue (Table 2-2) with prilocaine concentrations (19.67 $\mu g\ g^{-1}$) twice those of lignocaine (9.71 $\mu g\ g^{-1}$).

The observed lung uptake of lignocaine and prilocaine from this *in vitro* work is in agreement with the *in vivo* experiments of Åkerman et al (1966a) who, using rats, found that after intramuscular injections of both drugs, concentrations of prilocaine in lung tissue were much greater than those of lignocaine. This difference in lung uptake is however difficult to explain. Both drugs have the same pKa value (7.7; from Covino & Vassallo, 1976) so in the given pH range both drugs would be present in the same proportions of uncharged base and charged cation and hence equally available for tissue absorption. Of the two drugs, lignocaine is the more lipid soluble (Covino & Vassallo, 1976) suggesting that lignocaine should be more readily taken up by lung tissue. Prilocaine, however, is bound to plasma protein to a lesser degree than lignocaine (55% and 64% respectively; from Covino & Vassallo, 1976) so, assuming that both drugs are similarly bound to bovine serum albumin, more prilocaine than lignocaine would be unbound and available for lung absorption in greater amounts than lignocaine. This does not however adequately explain the two-fold difference in lung uptake of the two drugs.

TABLE 2-2

LUNG TISSUE CONCENTRATIONS OF LIGNOCAINE AND PRILOCAINE ± SD

(Values have been corrected for 100% efficiency of sample oxidation - efficiency of sample oxidiser = 95%)

	Lignocaine			Prilocaine
pH range	7.35 ⟶ 7.40	7.00 ⟶ 7.05	7.80 ⟶ 7.85	7.35 ⟶ 7.40
Lung tissue concentration ($\mu g\ g^{-1}$ wet tissue) ± SD	9.71 1.40	9.65	8.52	19.67 3.04
Number of lungs	6	2	2	6

The difference between lignocaine (pH 7.35 ⟶ 7.40) and prilocaine was statistically significant ($p < 0.001$, Student's t test for two means)

Using a lung slice incubation technique, Post et al (1979) showed that in rat lung slices lignocaine was accumulated to a greater extent at a pH of 8 than at normal pH (7.4) or acid pH (7.00). They proposed that this was due to greater amounts of local anaesthetic base existing at alkaline pH, which is much more lipid soluble than the cationic form of the drug and hence is more readily absorbed by lung tissue. However, Sjöstrand & Widman (1973), using an in vivo technique, demonstrated that acidotic rabbits attained bupivacaine levels in lung tissue five times greater than in normal rabbits. This they attributed to changes in the physical properties of tissue bound receptor sites, causing an increase in the affinity for bupivacaine. In this study, changing the perfusate pH from normal range (7.35 to 7.40) to both acidotic (7.00 to 7.05) and alkalotic (7.80 to 7.85) produced no perceptible changes of lignocaine concentrations in the perfusion medium (Table 2-1). Also there was little difference in the tissue drug concentrations (Table 2-2).

Feinstein & Paimre (1969) proposed that negatively charged groups on phospholipids and proteins of endothelial membranes could be responsible for the binding of the cationic form of local anaesthetics. So with the perfused lung preparation described here it is possible that at acid pH, less lignocaine base was absorbed by the tissue than at normal pH, but more was bound to the negative binding sites of the endothelial membranes and

vice versa at alkaline pH.

However, as only two preparations were run at each extreme of pH, no firm conclusion can be made from these results.

IN SITU PERFUSED RAT LUNG PREPARATIONS

INTRODUCTION

The previous experiments with isolated perfused rat lungs indicated the extent of lung uptake of lignocaine and prilocaine at steady state perfusate concentration. However, these results gave little indication of the ability of lung tissue to remove these drugs from the circulation in a single passage through the pulmonary vascular bed.

These experiments were designed to observe the effect on instantaneous bolus injections of a mixture of lignocaine and prilocaine in a single pass through the pulmonary vascular bed, to determine if the process of absorption was saturable and to see how these drugs effluxed from the lung.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing approximately 300 g were anaesthetised with approximately 40 mg pentobarbitone sodium given intraperitoneally and then the circulatory system heparinised by administering 500 IU heparin into a tail vein. A tracheotomy was performed on the animal by inserting a 4 cm length of polyethylene tubing into the trachea through a neck incision. A further incision was made in the abdomen of the rat and the aorta cut to kill the animal. The thorax was then opened to expose the heart and lungs.

Polyethylene tubing was used to catheterise the pulmonary artery via an incision in the right ventricle and the pulmonary venous return collected through a similar catheter introduced into the left atrium via an incision in the left ventricle. Both catheters were firmly sutured into position to prevent leakage of perfusion medium from the system. The lungs were perfused with Tyrodes buffer (Appendix 11) containing 4% bovine serum albumin (Fraction V, Sigma, Poole, Dorset, GB) and 0.1% glucose, since this was found to have a more stable pH under the conditions of this experiment than Kreb's buffer as used in the previous experiment.

The perfusion medium was adjusted to pH 7.35-7.40 at the start of the experiment using 0.1 molar hydrochloric acid or 0.1 molar sodium hydroxide as required and the pH was checked at the end of the experiment to ensure that no large fluctuations had occurred during the perfusion period. The lungs were partially inflated, the tracheotomy tube clamped and the perfusion flow adjusted to approximately 10 ml min^{-1} by altering the height of the perfusate reservoir. Perfusion flow was determined by measuring the perfusate volume collected over a 30 second period. The perfusate and the preparation were maintained at approximately 37^{o}C by warming both with a 60 watt light bulb.

The injection solutions were prepared by weighing out the appropriate amount of prilocaine hydrochloride

(Citanest, Astra Pharmaceutical Products Inc, Worcester, Mass, USA) and lignocaine hydrochloride monohydrate (Xylotox, Pharmaceutical Manufacturing Co, Bolton, GB) and dissolving the mixed drugs in less than 0.5 ml absolute ethanol (AR Grade, James Burrough Ltd, London, GB) then further diluting these solutions with distilled water. The same injection solution was used throughout all experiments at each concentration. Aliquots of each injection solution were assayed to verify their drug content.

Bolus injection of 10 µl drug solution (containing either 10, 50, 150, 250 or 1,000 µg of both drugs) into the system was achieved by inserting the needle of a 10 microlitre Hamilton syringe (Microliter No 701, Dyson Instruments, Durham, GB) through a rubber union connecting the pulmonary artery catheter to the perfusate reservoir. The injection was made over a period of less than one second.

The pulmonary venous outflow was collected continuously as 2 ml samples for ten samples then three further 10 ml samples were collected. Samples were analysed for lignocaine hydrochloride monohydrate and prilocaine hydrochloride by a gas liquid chromatographic technique as described in Appendix 1.

Experiments were also performed with just the two catheters joined together with perfusate flowing through the system to see if the catheters contributed to the

uptake of drug by the system.

RESULTS AND DISCUSSION

Perfusate Drug Concentration Data

At all doses, except the 1 mg dose, the perfusate concentrations of lignocaine were significantly greater than those of prilocaine for the initial samples (Table 2-3). The lignocaine concentrations then dropped more rapidly than the prilocaine concentrations (Fig 2-3) and the prilocaine concentrations became significantly greater than those of lignocaine until the end of the sampling period (Table 2-3). The drug concentration data in Fig 2-3 has been plotted at the mid-point volume of each sample as is appropriate for this type of sample collection and analysis.

The total amounts of each drug recovered by the end of the sampling period were calculated and have been expressed as a percentage of the initial dose (Table 2-4). There was a tendency with the 10 µg, 50 µg and 150 µg injections for lignocaine recoveries to be greater than for prilocaine. This tendency was reversed with the 250 µg and 1 mg injections. However, the difference between recoveries was only statistically significant with the 10 µg injection ($p < 0.001$).

TABLE 2-3

MEAN PERFUSATE DRUG CONCENTRATION DATA ±SD

Lig = Lignocaine hydrochloride monohydrate ($\mu g\ ml^{-1}$)

Pril = Prilocaine hydrochloride ($\mu g\ ml^{-1}$)

PLOTTED VOLUME (ml)	1	3	5	7	9	11	13	15	17	19	25	35	45
TOTAL VOLUME (ml)	2	4	6	8	10	12	14	16	18	20	30	40	50
INJECTION													
10 µg Lig	1.96†	1.36*	0.59	0.33*	0.21*	0.14*	0.10*	0.08*	0.06*	0.05*	0.02*		
±SD	0.48	0.29	0.12	0.07	0.04	0.03	0.02	0.02	0.01	0.02	0.01		
10 µg Pril	1.21	0.95	0.57	0.40	0.30	0.22	0.17	0.14	0.11	0.08	0.04		
±SD	0.37	0.15	0.10	0.08	0.06	0.04	0.04	0.03	0.02	0.02	0.01		
50 µg Lig	9.80*	5.99‡	2.44	1.39	0.85	0.56†	0.39‡	0.29*	0.22‡	0.18*	0.10‡	0.05†	0.03†
±SD	6.40	2.27	1.40	0.82	0.63	0.39	0.27	0.19	0.13	0.12	0.07	0.04	0.02
50 µg Pril	7.92	4.80	2.11	1.31	0.96	0.64	0.52	0.42	0.32	0.29	0.21	0.11	0.09
±SD	5.97	1.56	0.94	0.60	0.61	0.32	0.27	0.21	0.16	0.15	0.11	0.08	0.06

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TABLE 2-3 (continued)

PLOTTED VOLUME (ml)	1	3	5	7	9	11	13	15	17	19	25	35	45
TOTAL VOLUME (ml)	2	4	6	8	10	12	14	16	18	20	30	40	50
INJECTION													
150 µg Lig	42.65*	15.99‡	4.86	2.24*	1.33*	0.89*	0.64*	0.50*	0.40*	0.31*	0.17*	0.09*	0.05*
±SD	9.92	5.71	1.50	0.50	0.26	0.16	0.09	0.08	0.07	0.05	0.02	0.01	0.01
150 µg Pril	37.18	14.79	4.90	2.43	1.61	1.19	0.93	0.78	0.66	0.50	0.35	0.20	0.13
±SD	9.99	4.85	1.48	0.55	0.35	0.22	0.16	0.14	0.12	0.10	0.07	0.03	0.02
250 µg Lig	81.43†	28.26*	6.91*	2.86*	1.39*	1.03*	0.67*	0.51*	0.44*	0.31*	0.20*	0.10*	0.06*
±SD	14.96	9.96	1.91	0.72	0.41	0.33	0.13	0.12	0.16	0.06	0.07	0.02	0.02
250 µg Pril	78.96	29.97	8.04	3.57	1.77	1.50	1.04	0.82	0.79	0.61	0.39	0.21	0.13
±SD	16.33	10.34	2.15	0.83	0.43	0.44	0.22	0.17	0.26	0.15	0.13	0.04	0.04
1 mg Lig	328.49	95.41†	20.50*	8.72*	4.45*	2.92*	2.16*	1.66*	1.36‡	1.19	0.56*	0.29*	0.18*
±SD	39.93	12.97	4.87	1.68	1.08	0.65	0.48	0.48	0.42	0.70	0.12	0.06	0.04
1 mg Pril	332.97	100.87	23.80	10.71	5.64	3.92	2.95	2.22	1.84	1.48	0.92	0.52	0.30
±SD	39.61	14.07	4.43	2.03	1.31	0.94	0.50	0.49	0.39	0.28	0.24	0.10	0.05

Statistical differences between lignocaine and prilocaine concentrations (Student's paired t test)

† = $p < 0.05$ ‡ = $p < 0.01$ * = $p < 0.001$

FIGURE 2-3

INDIVIDUAL SEMILOGARITHMIC PLOTS OF MEAN PRILOCAINE AND LIGNOCAINE PERFUSATE CONCENTRATIONS .v. VOLUME OF PERFUSATE PASSING THROUGH RAT LUNGS, AFTER THE SIMULTANEOUS INJECTION OF EQUAL AMOUNTS OF EACH DRUG. (Statistical analysis of the data is given in Table 2-3)

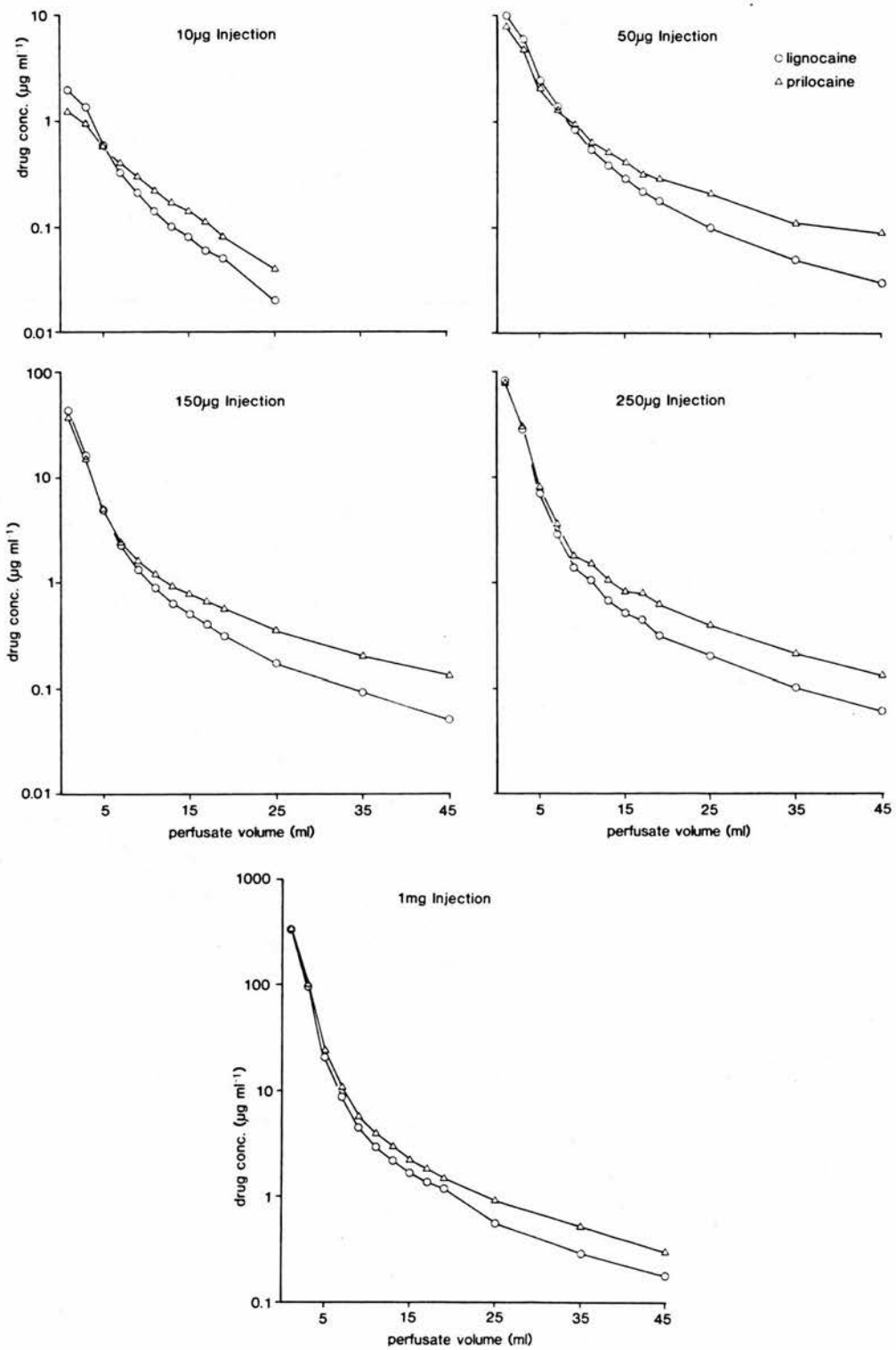


TABLE 2-4

% DRUG RECOVERED IN PERFUSATE SAMPLES
AT THE END OF THE SAMPLING PERIOD

(mean values ± SD)

	Dose (µg)	% Recovery	SD	p
Lignocaine	10	103.2	8.5	<0.001
Prilocaine	10	89.7	6.6	
Lignocaine	50	94.9	10.2	NS
Prilocaine	50	89.4	9.5	
Lignocaine	150	96.2	4.9	NS
Prilocaine	150	92.4	5.2	
Lignocaine	250	102.1	6.8	NS
Prilocaine	250	106.2	8.7	
Lignocaine	1000	96.8	6.8	NS
Prilocaine	1000	101.4	6.7	

Statistical analysis was by Student's paired t test

Pharmacokinetic Analysis

The semilogarithmic plot of drug concentration and perfusate volume can be likened to a plot of urinary excretion data where a semilogarithmic plot of excretion rate versus time is usually made. In this case, the time factor has been replaced by a volume factor and as a result of this, the excretion rate (usually expressed as amount of drug excreted per unit of time) becomes perfusate drug concentration, ie amount of drug 'excreted' per unit of volume.

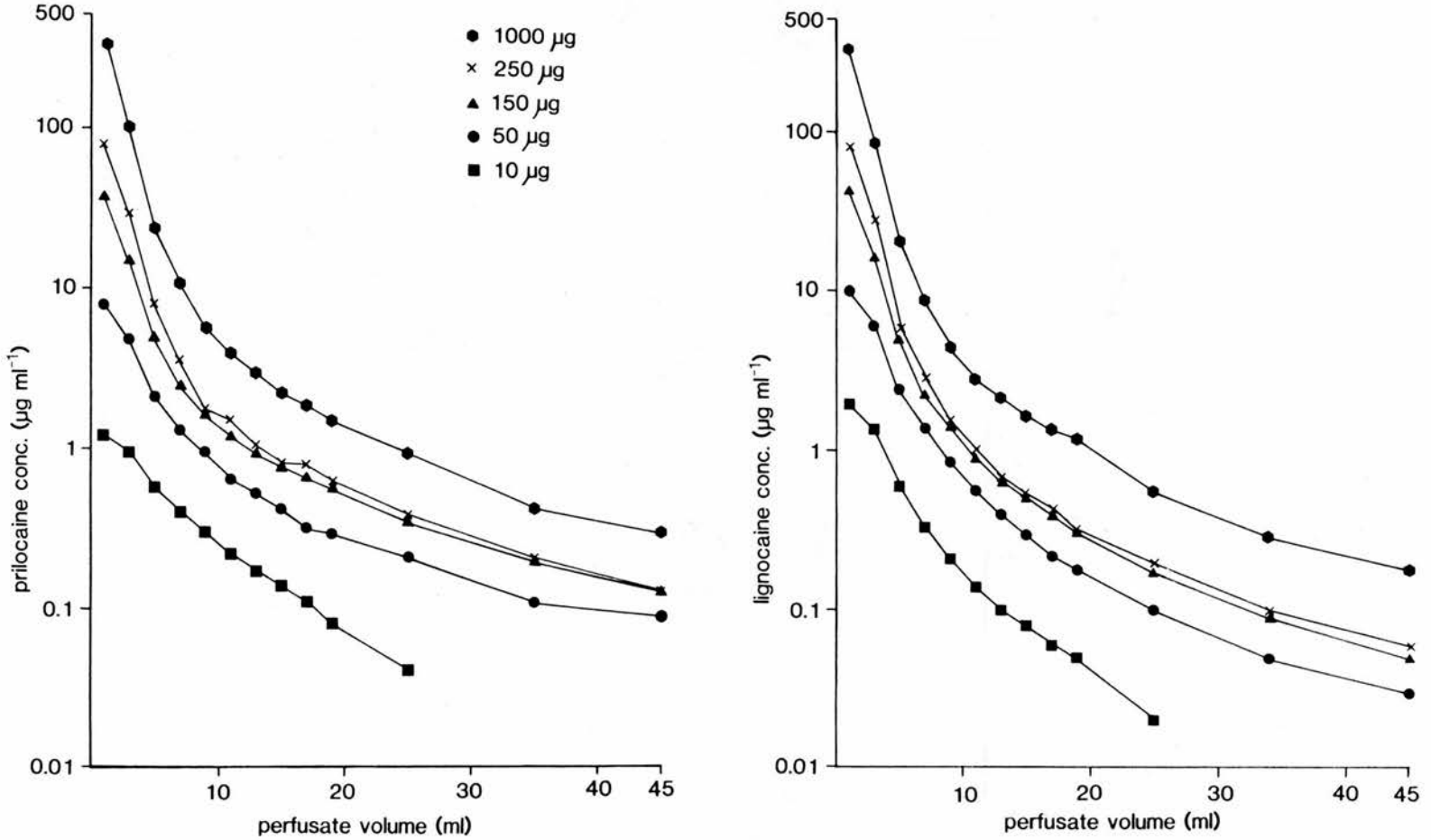
The perfusate concentration data from the 10 μg and 50 μg doses was found to have two components of efflux from the lung and the other doses were shown to be composed of three components (Fig 2-4). Because the x axis of the graph has been expressed as a volume, the half lives of the different phases have been expressed in millilitres, ie the volume of perfusate passing through the lung for the concentration of drug in the effluent perfusate to be halved ($V_{\frac{1}{2}}$). The terms for the slopes of these phases have accordingly been changed to millilitres^{-1}.

In all experiments, the first concentration data points (0-2 ml) were not included in the analysis as these samples contained the dead volume of the catheters and lungs, resulting in spurious drug concentration data.

The α phase of drug efflux from the lung (150 μg,

FIGURE 2-4

SEMILOGARITHMIC PLOTS OF MEAN PRILOCAINE PERFUSATE CONCENTRATIONS .v. PERFUSATE VOLUME AND MEAN LIGNOCAINE PERFUSATE CONCENTRATIONS .v. PERFUSATE VOLUME



250 µg and 1 mg doses only) was probably due to drug passing through the lungs unaffected by tissue binding or uptake. The $V_{\frac{1}{2}\alpha}$ values were very similar for both drugs (Table 2-5) with all values less than 1 ml.

The remaining two phases were attributed to two different sites of drug uptake in the lung tissue. Post et al (1978), in a study of lung extraction of lidocaine by isolated perfused rat lungs, have proposed at least two compartments of lignocaine uptake. Similarly, work with propranolol (Hayes & Cooper, 1971) has produced evidence for at least two types of binding site in rat lung, one having a high affinity for the drug and the other a low affinity for the drug.

The β and γ phases were probably related to two sites of drug uptake in the lung tissue, one site releasing the drugs back to the perfusate at a slower rate than the other.

Although the mean $V_{\frac{1}{2}\beta}$ values for prilocaine were greater than those for lignocaine at all doses except the 1 mg dose there was no statistical difference between these values. The $V_{\frac{1}{2}\gamma}$ values for prilocaine were greater than those for lignocaine at all doses and were significantly greater for the 50, 150 and 250 µg doses.

In studies with imipramine, amphetamine, chlorcyclizine and methadone, Anderson et al (1974), using

TABLE 2-5

PHARMACOKINETIC ANALYSIS OF PRILOCAINE AND LIGNOCAINE PERFUSATE CONCENTRATION DATA

(mean values ± SD)

LIGNOCAINE

Dose (µg)	10		50		150		250		1000	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
α (ml^{-1})					0.834	0.179	0.923	0.370	0.904	0.298
β (ml^{-1})	0.557	0.118	0.394	0.101	0.304	0.159	0.288	0.059	0.234	0.071
γ (ml^{-1})	0.123	0.016	0.080	0.009	0.059	0.011	0.065	0.005	0.056	0.006
$V_{\frac{1}{2}\alpha}$ (ml)					0.81	0.12	0.77	0.14	0.78	0.19
$V_{\frac{1}{2}\beta}$ (ml)	1.29	0.26	1.85	0.42	2.82	0.61	2.51	0.60	3.39	1.75
$V_{\frac{1}{2}\gamma}$ (ml)	5.71	0.75	8.79	1.02	12.00	4.90	10.65	0.83	12.44	1.28
A (µg ml^{-1})					183	107	351	160	1640	2070
B (µg ml^{-1})	5.16	2.03	17.77	16.26	7.99	4.42	13.05	7.79	29.93	23.31
C (µg ml^{-1})	0.53	0.19	0.76	0.47	0.78	0.29	1.01	0.27	2.06	0.50

continued overleaf

TABLE 2-5 (continued)

PRILOCAINE

Dose (µg)	10		50		150		250		1000	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
α (ml^{-1})					0.875	0.136	0.883	0.164	1.023	0.245
β (ml^{-1})	0.505	0.056	0.339	0.160	0.229	0.055	0.307	0.085	0.289	0.099
γ (ml^{-1})	0.119	0.018	0.066	0.028	0.051	0.006	0.057	0.007	0.055	0.011
$V_{\frac{1}{2}\alpha}$ (ml)					0.81	0.13	0.81	0.12	0.71	0.16
$V_{\frac{1}{2}\beta}$ (ml)	1.54	0.56	1.92	0.53	3.18	0.76	2.59	1.43	2.75	1.22
$V_{\frac{1}{2}\gamma}$ (ml)	5.93	0.94	11.72*	3.62	13.70*	1.60	12.35*	2.00	13.40	4.27
A ($\mu g\ ml^{-1}$)					161	87	318	194	1696	1082
B ($\mu g\ ml^{-1}$)	1.82	0.88	10.57	4.61	6.98	4.49	17.11	9.81	66.57	49.34
C ($\mu g\ ml^{-1}$)	0.83	0.24	0.91	0.26	1.29	0.43	1.68	0.46	3.83	1.41

* $p < 0.05$ for the difference between prilocaine and lignocaine data (Student's t test for two means)

an isolated perfused rabbit lung preparation, found the accumulation of basic amines in lung tissue to be dependent on the steady state blood concentration of the drug. They also found the accumulation of the drugs to be biphasic with a saturable and nonsaturable component. In this study, $V_{\frac{1}{2}\beta}$ and $V_{\frac{1}{2}\gamma}$ values increased with dose up to the 150 µg dose, the values thereafter all being very similar (Table 2-5). This data suggests that at a dose between 50 and 150 µg there is some saturation of the uptake sites of these two drugs. However, the fact that the terminal phase drug concentrations increase with dose indicates that the drug uptake in this phase is dose dependent. These results could be interpreted as the site of uptake for the γ phase being intracellular absorption of the unionised form of the drug and that the β component could be related to extracellular binding of the cationic form of the drug to negatively charged binding sites of the endothelial cell membranes.

S E C T I O N 3

METABOLISM OF PRILOCAINE AND LIGNOCAINE BY KIDNEY AND LUNG SLICE PREPARATIONS

LUNG AND KIDNEY TISSUE INCUBATIONS WITH LIGNOCAINE AND PRILOCAINE

INTRODUCTION

Metabolism of prilocaine by rat kidney slices was demonstrated by Geddes (1965). Åkerman et al (1966a) found that both kidney and lung slices from cats and rabbits were capable of prilocaine metabolism but had little effect on lignocaine.

In the human volunteer study (Section 1) it had been proposed that the lung, and possibly the kidneys, could be the site of extra-hepatic clearance of prilocaine. These experiments were designed to determine the ability of lung and kidney slices from two species of animal, dog and rabbit, used in work described later in this thesis, to metabolise both lignocaine and prilocaine.

MATERIALS AND METHODS

Lung and kidney samples were obtained from New Zealand White rabbits and mongrel dogs. Once the tissues were removed they were placed on ice and incubations were started within 45 minutes.

Slices of tissue, 1-2 mm thick, were cut from the main piece of tissue using a scalpel. Three slices of each tissue, weighing approximately 2 g for kidney

samples and 1 g for lung samples, were placed in separate weighed plastic 50 ml containers with screw cap lids. To each flask was added 10 ml of oxygenated Kreb's buffer (Appendix 10) with 0.1% glucose added which had previously been adjusted to a pH of 7.40. There were three incubation periods of 0, 30 and 60 minutes with one container for each incubation period.

Lignocaine and prilocaine were introduced to the tissue samples by two methods. In three of the five rabbit tissue preparations, and one of the four dog tissue preparations, an intravenous injection of a mixture of the two drugs was given prior to sacrificing the animal and removing the required tissue. With the remaining tissue preparations, a mixture of the two drugs in aqueous solution was added to the incubation medium at the start of the incubation period. Using the latter technique, the resultant concentration of the drugs in the system was approximately 10 $\mu g\ ml^{-1}$.

As a control, 1 ml of 1 molar hydrochloric acid was immediately added to the first flask of each set of three and these were frozen. The lids of the other two flasks of each set were sealed and these placed in a water bath with a reciprocal shaker at $37^{o}C$. After 30 minutes in the water bath, the second flask in each series was removed and 1 ml of 1 molar hydrochloric acid added to each and the flasks placed in a freezer. The same procedure was repeated with the remaining flasks after a total incubation period of 60 minutes.

At a later date, the flasks were removed from the freezer and allowed to thaw. The flasks and contents were then reweighed to determine the total weight of the incubation medium plus tissue. The contents of the flasks were homogenised for a period of one minute (Ultra-Turrax, Scottish Scientific Instruments, Kingsknowe, Edinburgh, GB) and aliquots of the homogenate analysed for lignocaine and prilocaine content, using a gas liquid chromatographic technique adapted from the method described for rat lung perfusate in Appendix 1.

Flasks containing drug and incubation medium, but no tissue, were run through the same procedure as described above to act as control samples to monitor the effect of the incubation process on drug concentrations.

RESULTS AND DISCUSSION

The results have been expressed as the percentage of drug remaining in the samples after the incubation period and have been corrected for the tissue content of each sample.

With tissue removed from animals injected with local anaesthetic prior to organ removal there appeared to be a tendency for a higher percentage of drug metabolism. This however could have been due to the fact that the overall sample drug concentrations with these preparations was less than those of the preparations to which drug was

added to the incubation medium. Kidney samples of both species were unable to metabolise lignocaine but had a small capability for prilocaine metabolism (Fig 3-1).

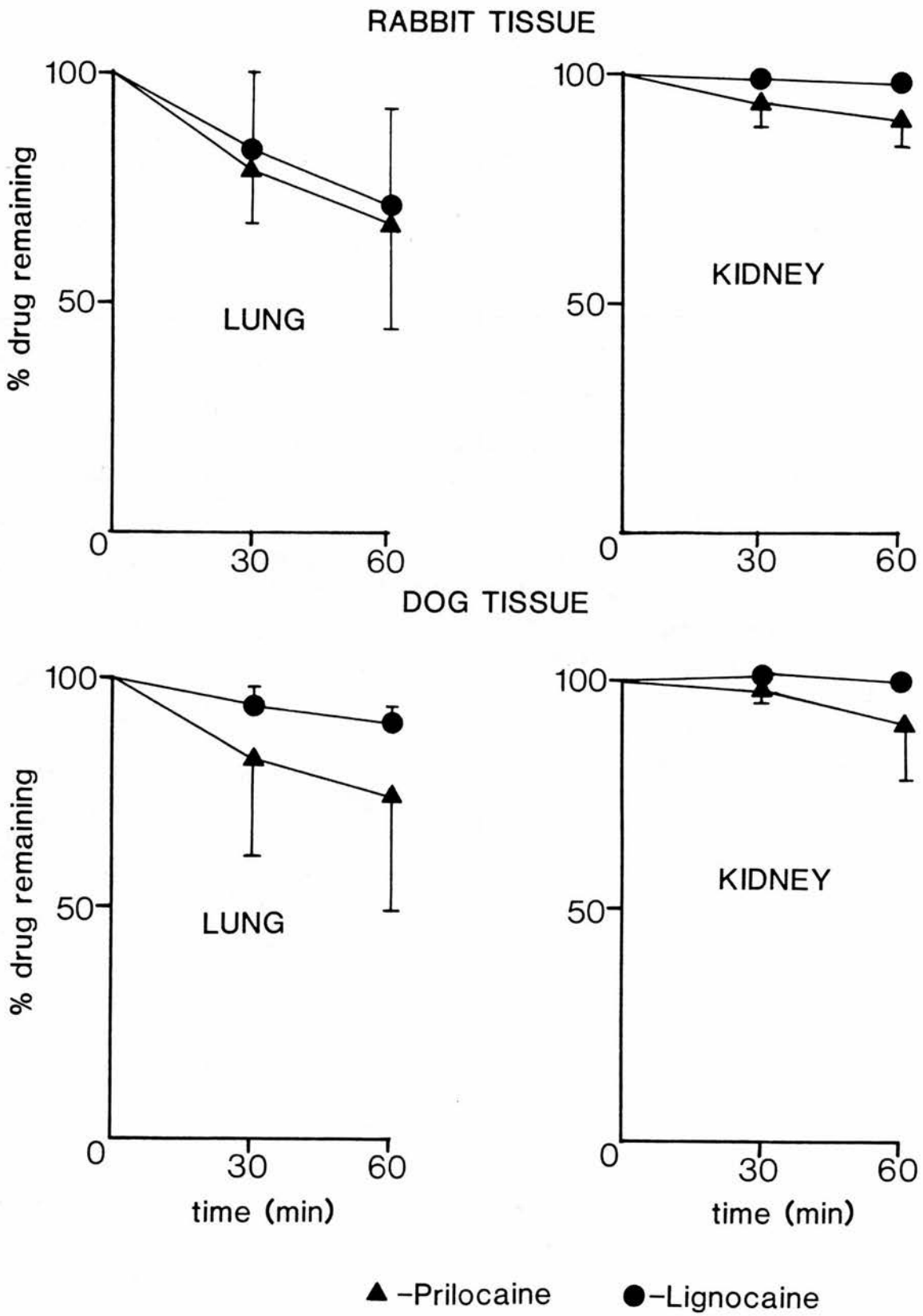
Rabbit lung slices appeared to metabolise both prilocaine and lignocaine to the same extent, but dog lung slices appeared to have a greater ability to metabolise prilocaine compared to lignocaine (Fig 3-1). Blank incubations, with no tissue in the perfusate/drug mixture, showed no loss of either drug from the system over the 60 minute incubation period.

These results are similar to those obtained by Åkerman et al (1966a) in that both studies showed that rabbit lung slices were capable of metabolising prilocaine. However, unlike the data presented here, they did not observe any metabolism of lignocaine by rabbit lung slices. The data from rabbit kidney slice incubations obtained here was in agreement with the results of Åkerman et al, except that the rabbit kidney slice incubation data presented here showed a lower capability for prilocaine metabolism. Geddes (1965) had also demonstrated prilocaine metabolism by rat kidney slice incubation.

Åkerman et al also used cat tissue for their incubation studies. Cat lung and kidney tissue slices were found to be capable of metabolising both prilocaine and lignocaine, although prilocaine was metabolised to a greater extent than lignocaine in both tissues. In the present study, dog lung tissue was used and shown to metabolise more

FIGURE 3-1

GRAPHS TO ILLUSTRATE THE AMOUNTS OF PRILOCAINE AND LIGNOCAINE METABOLISED BY LUNG AND KIDNEY TISSUE SLICES FROM RABBITS AND DOGS
(Mean values ± SD bars)



prilocaine than lignocaine while kidney tissue, although capable of some prilocaine metabolism, showed no lignocaine metabolism. Post et al (1978) had been unable to find any metabolism of ^{14}C-lignocaine by rat lung slice incubations.

Care should be taken however in placing too much importance in these results as other workers have reported that lung tissue preparations have been capable of chlorcyclizine and imipramine metabolism but this has not been true for intact perfused lung preparations (Orton et al, 1973; Law et al, 1974; Eling et al, 1975). The reason for this is probably due to the large number of different cell types in the lung, many of which will not absorb large quantities of drug from the blood in an intact lung, and it may well be that enzymes released from these cells by slicing or homogenising are the cause of some drug metabolism.

However, the data from these lung and kidney slice incubations suggested that perhaps the lung was the most likely place to look for extra-hepatic metabolism of prilocaine.

S E C T I O N 4

PULMONARY EXTRACTION, HEPATIC EXTRACTION AND PHARMACOKINETICS OF PRILOCAINE IN THE ANAESTHETISED DOG

PHARMACOKINETICS AND PULMONARY EXTRACTION OF PRILOCAINE IN DOGS

INTRODUCTION

In the pharmacokinetic study of prilocaine in human volunteers, it was proposed that the lung may be a site of extra-hepatic metabolism of prilocaine.

The work with isolated perfused rat lungs showed a significant lung uptake of prilocaine. Furthermore, the *in vitro* experiments with lung slice preparations demonstrated prilocaine was metabolised by lung tissue, confirming the results obtained by Åkerman et al (1966a).

This experiment was designed to describe the pharmacokinetics of prilocaine in the dog and to determine to what extent pulmonary metabolism contributed to the total body clearance of this drug.

MATERIALS AND METHODS

The Animals and Anaesthesia

Six mongrel dogs in the weight range 9.5 - 18.0 kg (Centre for Laboratory Animals, Bush Estate, Edinburgh, GB) were used in this experiment. The animals were fasted overnight, premedicated with promazine (2 mg) and atropine (0.6 mg) given intramuscularly (approximately 1 hr before induction of anaesthesia), then anaesthetised with pentobarbitone sodium given intravenously (approximately

25 mg kg^{-1}). Endotracheal intubation was performed and anaesthesia maintained by spontaneous respiration of a mixture of nitrous oxide (3 l min^{-1}) and oxygen (1 l min^{-1}) through an open anaesthetic circuit.

The Preparation

A catheter was inserted into the right femoral artery to monitor arterial blood pressure, using a Sanborn transducer (Hewlett Packard Ltd, South Queensferry, West Lothian, GB) giving both a calibrated visual display and chart recording. The arterial blood pressure and the heart rate were determined from the chart recording. The left carotid artery was exposed by an incision in the neck and a catheter passed through the artery into the aortic arch for the withdrawal of blood samples. In the first three dogs it was hoped to obtain mixed central venous blood samples from a catheter passed through the right jugular vein and into the right atrium. In the last three dogs, this method was improved by using a Swan-Ganz catheter (American Hospital Supply (UK) Ltd, Didcot, Oxon, GB) introduced by the same route and positioned in the pulmonary artery using blood pressure tracing to verify its position.

A vein in the right forepaw of the animal (right cephalic vein) was cannulated (Medicut, 18 G, Sherwood Medical Industries, Ballymoney, Co Antrim, N Ireland) for the infusion of prilocaine hydrochloride (Citanest, Astra Pharmaceuticals Inc, Worcester, Mass, USA).

All catheters, unless otherwise stated, were prepared from Portex manometer tubing (Portex Ltd, Hythe, Kent, GB) and the two sampling catheters were designed to have the same dead volume. The correct positioning of all the catheters was verified by autopsy at the end of each experiment.

The Infusion

All six dogs received a 100 minute infusion of a prilocaine hydrochloride solution estimated to obtain a steady state blood concentration of approximately 2 $\mu g\ ml^{-1}$ using the formula:

$$k_o = C_{ss}\ \beta\ V_B$$

where k_o = the rate of delivery of drug into the system ($mg\ min^{-1}\ kg^{-1}$);

C_{ss} = the steady state concentration of prilocaine hydrochloride ($\mu g\ ml^{-1}$);

β = the slope of the elimination phase of the drug (min^{-1});

and V_B = the total volume of distribution of the drug ($l\ kg^{-1}$)

Pharmacokinetic data (β and V_B) from the human volunteer study (see Table 1-2) was substituted in the above equation to give an approximation of the infusion rate k_o. The resulting infusion rate was 0.075 $mg\ min^{-1}\ kg^{-1}$ and, having been informed that the animals to be

used would weigh approximately 20 kg, an infusion rate of 1.6 mg min^{-1} was chosen. As the predicted time to reach steady state blood concentrations with a constant rate infusion was over 7.5 hours (5 x $t_{\frac{1}{2}\beta}$, using the $t_{\frac{1}{2}\beta}$ value from the human volunteer study) a loading infusion of approximately 40% of the total dose was given to reduce the time to reach reasonably constant blood concentrations to 60 minutes.

A solution of prilocaine (5 mg ml^{-1}) was diluted to a concentration of 4 mg ml^{-1} with Hartmann's Solution (Compound Sodium Lactate Injection BP, Travenol Laboratories Ltd, Thetford, Norfolk, GB) and infused through the cannula in the right cephalic vein using a Harvard syringe infusion pump (Harvard Apparatus, Millis, Mass, USA) at a rate of 1 ml min^{-1} (4 mg min^{-1}) for 20 minutes then 0.4 ml min^{-1} (1.6 mg min^{-1}) for a further 80 minutes to give a total infusion volume of 52 ml containing 208 mg prilocaine hydrochloride.

The Samples

In all experiments, blood samples were withdrawn simultaneously from the aortic and the right atrial/ pulmonary artery catheters at regular intervals both during and after the drug infusion (Table 4-1). The technique involved withdrawing blood in excess of the dead volume of the catheters then using new syringes to withdraw each 3 ml sample. The dead volume blood was then returned to the preparation through the sampling

TABLE 4-1

EXPERIMENTAL PROCEDURE AND SAMPLING TIMES

Anaesthesia and catheter insertion	-45 min $\longrightarrow$ 0 min
Prilocaine hydrochloride infusion	0 $\longrightarrow$ 20 min at 4 mg min^{-1} 20 $\longrightarrow$ 100 min at 1.6 mg min^{-1}
Blood samples (both sampling sites)	0, 20, 40, 60, 62, 64, 66, 68, 70, 72, 76, 78, 80, 82, 84, 86, 88, 90, 95, 100, 102, 104, 106, 108, 110, 115, 120, 130, 145, 160, 190, 220, 250, 280 min
Blood gases	0, 60, 100, 130, 220 min
Halothane 1.5% (dogs 1 and 2 only)	75 $\longrightarrow$ 87 min

catheters and these were flushed with 3 ml heparinised Hartmann's solution (10 units heparin ml^{-1}) to replace blood loss and to keep the catheters patent.

The blood samples were transferred to 10 ml plastic lithium heparin tubes (Brunswick, Sherwood Medical Ltd, Ballymoney, Co Antrim, N Ireland) and refrigerated until assayed for whole blood prilocaine hydrochloride content using a GLC technique described in Appendix 1.

Blood samples were also withdrawn at regular intervals from the aortic catheter into heparinised glass syringes for blood gas analysis (Corning 175 Automatic pH/blood gas System, Halstead, Essex, GB).

In dogs 1 and 2, 1.5% halothane (Fluothane, ICI Pharmaceuticals, Macclesfield, Cheshire, GB) was added to the nitrous oxide/oxygen mixture during the infusion period (75-85 minutes) to see what effect the addition of a volatile anaesthetic gas would have on prilocaine blood concentrations.

The whole blood prilocaine concentration data was subjected to pharmacokinetic analysis on the basis of a two compartment open model as described previously (see Section 1) and statistical analysis of the results was carried out using Student's t test for paired and unpaired data where appropriate.

RESULTS AND DISCUSSION

Heart Rate, Blood Pressure and Blood Gases Appendices 12 and 13

During the 100 minutes of the infusion the heart rate was virtually unchanged being 159 beat min^{-1} at the start of the infusion, 150 beat min^{-1} at the end of the infusion and 142 beat min^{-1} 120 minutes after the end of the infusion.

No remarkable changes in blood pressure were observed throughout the experiments except during the period of halothane administration in dogs 1 and 2 where a decrease in both diastolic and systolic pressures were observed. In these two animals, blood pressure returned to normal shortly after the termination of the halothane administration.

Blood gas analysis showed that all the dogs were acidotic and hypercarbic for the duration of the experiments compared to normal values (from Feigl & d'Alecy, 1972; Appendix 13), as would be expected in spontaneously breathing anaesthetised animals. However, all animals maintained a fairly constant blood gas status through the course of each experiment.

Prilocaine Blood Concentration Data

For dogs 1, 2 and 3, the prilocaine blood concentration data obtained from right atrial samples were found to be erratic. When the areas under the curves were measured, the AUC_M for the aorta sample data

of dog 2 was much greater than the value for the right atrial data with the reverse situation occurring with dog 3 (Table 4-2). With the other preparations the AUC_M values were very similar. It is probable that the differences in the results for dogs 2 and 3 were due to incomplete mixing of venous blood in the right atrium. Because of this, it was decided to exclude the pharmacokinetic data obtained from dogs 1, 2 and 3.

For dogs 4, 5 and 6 in which mixed venous blood was sampled from a Swan-Ganz catheter in the pulmonary artery, the mixed venous and arterial prilocaine concentration/time profiles were found to be very similar (Fig 4-1). However, the mixed venous concentrations were statistically significantly higher than the arterial concentrations at steady state in dogs 4 and 6 (Table 4-3). With dog 5, the steady state concentrations at both sampling sites were almost identical. From the prilocaine concentration data in Table 4-3 it is apparent that the drug concentrations continued to rise slowly between 60-100 minutes, ie absolute steady state concentrations (C_{ss}) had not been attained. This is further reflected by a comparison of the mean C_{ss} values and the predicted C_{ss} values (Table 4-4) obtained using the formula:

$$C_{ss} = \frac{k_o}{\beta . V_B}$$

The prilocaine concentrations at 100 minutes (Table 4-4) had, however, almost reached the predicted C_{ss} values.

TABLE 4-2

COMPARISON OF AUC_M VALUES FOR BOTH ARTERIAL AND MIXED VENOUS PRILOCAINE CONCENTRATION DATA

		Preparation					
		1	2	3	4	5	6
AUC_M ($\mu g\ ml^{-1}.min$)	Arterial	352	382	521	538	364	468
	Mixed Venous	333	241	772	539	370	467

FIGURE 4-1

INDIVIDUAL SEMILOGARITHMIC PLOTS OF PRILOCAINE BLOOD CONCENTRATION .v. TIME FOR DOGS 4, 5 & 6

X = arterial concentrations

O = mixed venous concentrations

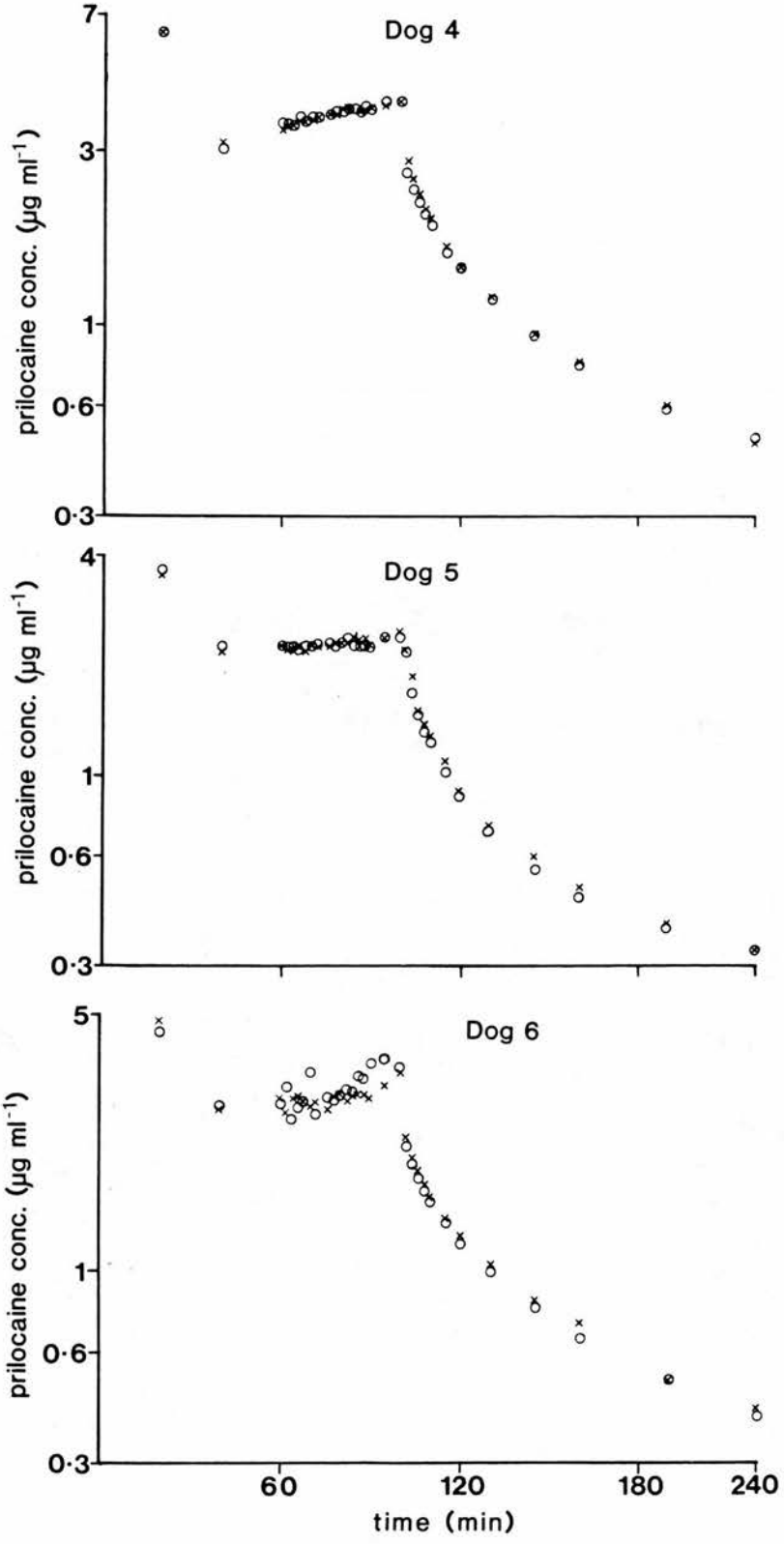


TABLE 4-3

STEADY STATE PRILOCAINE HYDROCHLORIDE CONCENTRATIONS AT STEADY STATE (60-100 MIN) FOR DOGS 4, 5 AND 6

($\mu g\ ml^{-1}$)

TIME (min)	DOG 4		DOG 5		DOG 6	
	ART	MV	ART	MV	ART	MV
60	3.39	3.52	2.25	2.26	2.92	2.81
62	3.45	3.52	2.18	2.22	2.68	3.17
64	3.48	3.48	2.18	2.24	2.95	2.57
66	3.60	3.69	2.27	2.19	2.97	2.76
68	3.59	3.57	2.18	2.27	2.86	2.87
70	3.61	3.68	2.27	2.24	2.80	3.45
72	3.68	3.69	2.26	2.28	2.88	2.61
76	3.72	3.74	2.24	2.30	2.73	2.95
78	3.76	3.82	2.27	2.22	2.98	2.89
80	3.85	3.80	X	2.30	3.02	2.95
82	3.92	3.91	2.26	2.39	2.87	3.09
84	X	3.91	2.37	2.25	2.99	3.06
86	3.84	3.80	2.31	2.22	3.00	3.39
88	3.89	3.92	2.35	2.25	3.00	3.30
90	3.90	3.86	2.27	2.23	2.91	3.64
95	3.94	4.04	2.35	2.38	3.18	3.78
100	4.05	4.08	2.47	2.39	3.44	3.57
$\bar{x}$	3.72	3.78	2.28	2.27	2.95	3.11
SD	0.20	0.18	0.08	0.06	0.17	0.36
p	<0.05		NS		<0.05	

ART = Arterial concentrations
MV = Mixed venous concentrations
X = No sample obtained

Statistical analysis was by Student's paired t test

TABLE 4-4

COMPARISON OF THE PREDICTED AND MEAN C_{ss} VALUES AND C AT 100 MINUTES (THE END OF THE INFUSION) OF PRILOCAINE HYDROCHLORIDE ($\mu g\ ml^{-1}$)

	DOG 4		DOG 5		DOG 6	
	ART	MV	ART	MV	ART	MV
Predicted C_{ss}	4.14	4.14	2.80	2.80	3.60	3.60
Mean C_{ss}	3.72	3.78	2.28	2.27	2.95	3.11
C at 100 min	4.05	4.08	2.47	2.39	3.44	3.57

ART = Arterial concentrations
MV = Mixed venous concentrations

After terminating the infusion, arterial prilocaine concentrations were consistently greater than mixed venous concentrations in these three preparations (Table 4-5). This was probably due to prilocaine, taken up by the lung tissue during the infusion, returning to the circulation from the lung tissue to maintain the tissue/blood equilibrium as prilocaine was removed from the body by metabolism.

Returning to the data for dogs 1 and 2, the introduction of halothane (1.5%) to the anaesthetic gas mixture caused both arterial and right atrial prilocaine concentrations to rise (Appendix 14). Hughes et al (1980) demonstrated that 2% halothane reduced the total hepatic blood flow in the dog by 40% as well as reducing the cardiac output. These factors would lead to a reduced clearance of drugs metabolised by the liver and would account for the increases in prilocaine blood concentrations found here.

Pharmacokinetic Analysis

The pharmacokinetic analysis of the prilocaine concentration data was based on a two compartment open model as described in Section 1. Because the infusion was given at two different rates, it was not possible to determine the corrected A and B intercept values using the equations of Loo & Riegelman (1970), as the rate of the infusion is not taken into account with this method. The derivation of the equations used to

TABLE 4-5

POST INFUSION WHOLE BLOOD PRILOCAINE CONCENTRATION DATA

($\mu g\ ml^{-1}$)

TIME (min)	DOG 4		DOG 5		DOG 6	
	ART	MV	ART	MV	ART	MV
102	2.80	2.60	2.18	2.17	2.29	2.17
104	2.49	2.32	1.82	1.66	2.02	1.95
106	2.27	2.16	1.49	1.46	1.86	1.77
108	2.07	2.00	1.35	1.30	1.70	1.65
110	1.96	1.87	1.26	1.23	1.58	1.56
115	1.62	1.57	1.08	1.01	1.37	1.33
120	1.43	1.42	0.90	0.87	1.23	1.18
130	1.19	1.17	0.72	0.70	1.03	0.98
145	0.94	0.94	0.59	0.55	0.82	0.79
170	0.79	0.78	0.49	0.46	0.71	0.65
190	0.60	0.58	0.39	0.38	0.50	0.50
220	0.48	0.49	0.33	0.33	0.42	0.40
p	<0.01		<0.01		<0.001	

ART = Arterial concentrations
MV = Mixed venous concentrations

p values for difference between ART and MV (Student's paired t test)

determine these values is now given.

Derivation of A and B Intercept Values for an Infusion Given at Two Rates

In the method shown here, the kinetics of each infusion rate have been considered separately and the resultant formulae have been derived from a summation of the characteristics of the two separate infusions.

Fig 4-2a represents an actual concentration/time profile resulting from the combined infusion and Fig 4-2b represents the separated time/concentration profiles for the two infusions superimposed on the same graph.

For the first infusion for times $t > T_1$:

$$C_1t = Re^{-\alpha(t-T_1)} + Se^{-\beta(t-T_1)} \qquad 1$$

where $$R = \frac{k_o(\alpha-k_{21})(1-e^{-\alpha T_1})}{V_c\alpha(\alpha-\beta)} \qquad 2$$

and $$S = \frac{k_o(k_{21}-\beta)(1-e^{-\beta T_1})}{V_c\beta(\alpha-\beta)} \qquad 3$$

For the second infusion for times t (where $t > T_2$):

$$C_2t = R'e^{-\alpha(t-T_2)} + S'e^{-\beta(t-T_2)} \qquad 4$$

where $$R' = \frac{k_o'(\alpha-k_{21})(1-e^{-\alpha(T_2-T_1)})}{V_c\alpha(\alpha-\beta)} \qquad 5$$

FIGURE 4-2

a STYLISED SEMILOGARITHMIC PLOT OF DRUG CONCENTRATION .v. TIME AFTER AN INFUSION GIVEN AT TWO DIFFERENT RATES

b SUPERIMPOSED SEMILOGARITHMIC PLOTS OF DRUG CONCENTRATION .v. TIME AS A RESULT OF TWO SEPARATE INFUSIONS GIVEN AT DIFFERENT RATES

GLOSSARY OF SYMBOLS USED IN THE DERIVATION OF THE FORMULA TO OBTAIN CORRECTED A & B VALUES FROM DRUG CONCENTRATION DATA OBTAINED FROM AN INFUSION GIVEN AT TWO DIFFERENT RATES

T_1 & T_2 = Times to the end of the first and second infusions (min)

C = Blood drug concentration ($\mu g\ ml^{-1}$)

k_o = First infusion rate ($mg\ min^{-1}$)

k_o' = Second infusion rate ($mg\ min^{-1}$)

A' & B' = Calculated intercept values of the α & β phases respectively after the combined infusion ($\mu g\ ml^{-1}$)

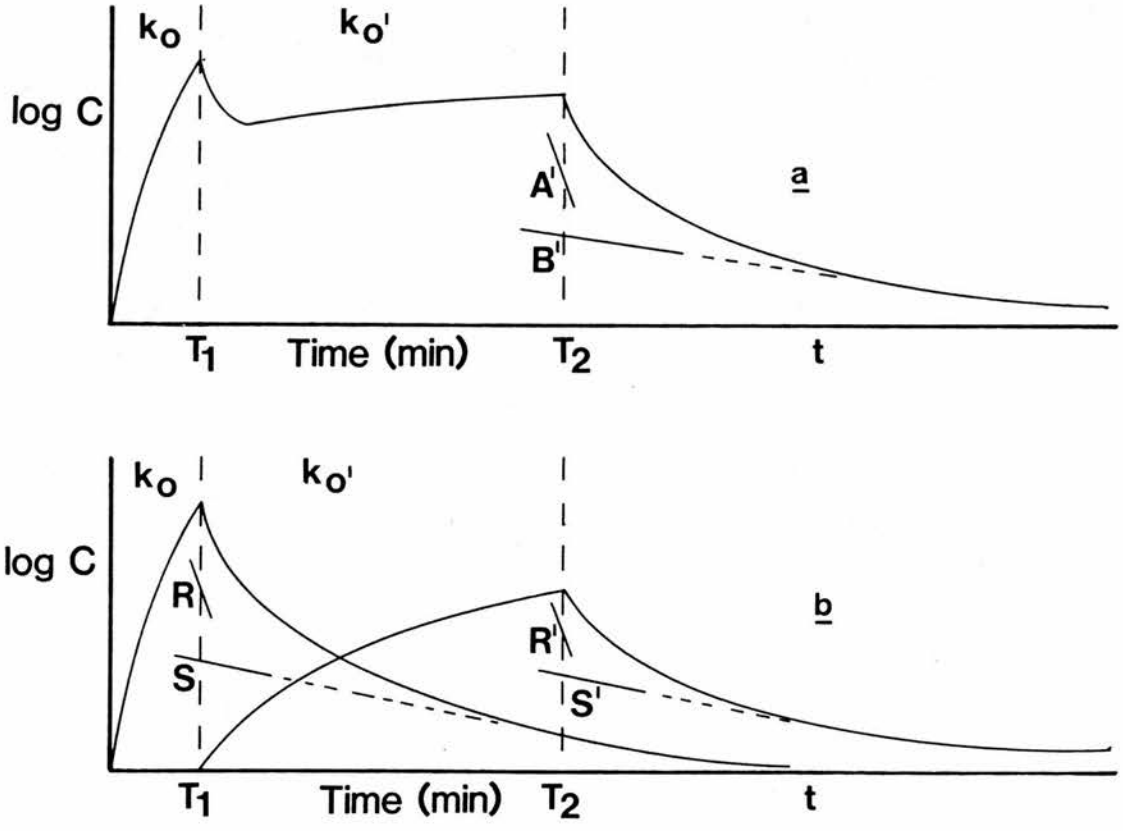
R & S = Hypothetical intercept values at T_1 of the α &β phases of the first infusion curve ($\mu g\ ml^{-1}$)

R' & S' = Hypothetical intercept values at T_2 of the α & β phases of the second infusion curve ($\mu g\ ml^{-1}$)

t = Time after the end of the infusion (min)

X_o = The total dose of drug administered (mg)

α, β, k_{10}, k_{21}, k_{12} & V_c are as previously defined in Section 1



$$\text{and} \quad S' = \frac{k_o'(k_{21}-\beta)(1-e^{-\beta(T_2-T_1)})}{V_c\beta(\alpha-\beta)} \qquad 6$$

so combining 1 and 4 where $C_1 + C_2 = C$ then:

$$C = Re^{-\alpha(t-T_1)} + R'e^{-\alpha(t-T_2)} + Se^{-\beta(t-T_1)} + S'e^{-\beta(t-T_2)} \qquad 7$$

when $t = T_2$ then:

$$C = Re^{-\alpha(T_2-T_1)} + R'e^{-\alpha(o)} + Se^{-\beta(T_2-T_1)} + S'e^{-\beta(o)} \qquad 8$$

so:

$$C = Re^{-\alpha(T_2-T_1)} + R' + Se^{-\beta(T_2-T_1)} + S' \qquad 9$$

At the termination of the infusion at $t = T_2$:

$$C = A'e^{-\alpha(t-T_2)} + B'e^{-\beta(t-T_2)} \qquad 10$$

as $t-T_2 = O$, then at $t = T_2$, $C = A' + B'$ 11

$$\text{where} \quad A' = Re^{-\alpha(T_2-T_1)} + R' \qquad 12$$

$$\text{and} \quad B' = Se^{-\beta(T_2-T_1)} + S' \qquad 13$$

Substituting equations 2 and 5 in 12 for R and R' then:

$$A' = \left[\frac{k_o(\alpha-k_{21})(1-e^{-\alpha T_1}).e^{-\alpha(T_2-T_1)}}{V_c\alpha(\alpha-\beta)} + \frac{k_o'(\alpha-k_{21})(1-e^{-\alpha(T_2-T_1)})}{V_c\alpha(\alpha-\beta)}\right]$$

$$A' = \frac{\alpha - k_{21}}{V_c \alpha (\alpha - \beta)} \left[k_o (1 - e^{-\alpha T_1}) . e^{-\alpha (T_2 - T_1)} + k_o' (1 - e^{-\alpha (T_2 - T_1)}) \right]$$

$$A' = \frac{\alpha - k_{21}}{V_c \alpha (\alpha - \beta)} \left[k_o . e^{-\alpha (T_2 - T_1)} - k_o e^{-\alpha T_1} . e^{-\alpha (T_2 - T_1)} \right.$$

$$\left. + k_o' - k_o' . e^{-\alpha (T_2 - T_1)} \right] \qquad 14$$

Also substituting equations 3 and 6 in 13 for S and S' then:

$$B' = \left[\frac{k_o (k_{21} - \beta) (1 - e^{-\beta T_1}) . e^{-\beta (T_2 - T_1)}}{V_c \beta (\alpha - \beta)} \right.$$

$$\left. + \frac{k_o' (k_{21} - \beta) (1 - e^{-\beta (T_2 - T_1)})}{V_c \beta (\alpha - \beta)} \right]$$

$$B' = \frac{k_{21} - \beta}{V_c \beta (\alpha - \beta)} \left[k_o . e^{-\beta (T_2 - T_1)} - k_o . e^{-\beta T_1} . e^{-\beta (T_2 - T_1)} \right.$$

$$\left. + k_o' - k_o' . e^{-\beta (T_2 - T_1)} \right] \qquad 15$$

At this point it is necessary to substitute actual data into the equations:

eg dog 7 . $k_o = 4$ mg min^{-1}

$k_o' = 1.6$ mg min^{-1}

$\alpha = 0.0803\ min^{-1}$

$\beta = 0.0089\ min^{-1}$

$T_1 = 20$ min

$$T_2 - T_1 = 80 \text{ min}$$

$$A' = 1.15 \ \mu g \ ml^{-1}$$

$$B' = 1.14 \ \mu g \ ml^{-1}$$

So equation 14 becomes:

$$1.15 = \left[4.e^{-0.0803.80} - 4.e^{-0.0803.20} .e^{-0.0803.80} + 1.6 - 1.6.e^{-0.0803.80}\right] \times \frac{(0.0803-k_{21})}{V_c.0803(0.0803-0.0089)}$$

$$1.15 = \frac{(0.0803-k_{21})}{0.00573V_c} \left[0.00649 - 0.00130 + 1.6 - 0.00260\right]$$

$$1.15 = \frac{(0.0803-k_{21})}{0.00573V_c} \cdot 1.6026$$

$$1.15 = \frac{0.1287-1.6026k_{21}}{0.00573V_c}$$

$$V_c = \frac{0.1287-1.6026k_{21}}{0.00659} \qquad 16$$

$$0.00659 \ V_c = 0.1287-1.6026k_{21} \qquad 17$$

Equation 15 now becomes:

$$1.14 = \frac{(k_{21}-0.0089)}{V_c 0.0089(0.0803-0.0089)} \left[4.e^{-0.0089.80} - 4.e^{-0.0089.20} .e^{-0.0089.80} + 1.6 - 1.6e^{-0.0089.80}\right]$$

$$1.14 = \frac{(k_{21}-0.0089)}{0.000636V_c} \left[1.96 - 1.64 + 1.6 - 0.785\right]$$

$$1.14 = \frac{(k_{21}-0.0089)}{0.000636V_c} \cdot 1.135$$

$$1.14 = \frac{1.135k_{21}-0.0101}{0.000636V_c}$$

$$V_c = \frac{1.135k_{21}-0.0101}{0.000725} \qquad 18$$

$$0.000725\ V_c = 1.135k_{21}-0.0101 \qquad 19$$

Substituting 18 for V_c in 17:

$$0.00659 \frac{1.135k_{21}-0.0101}{0.000725} = 0.1287 - 1.6026k_{21}$$

$$0.00748k_{21} - 0.0000666 = 0.0000933 - 0.00116k_{21}$$

$$0.00748k_{21} + 0.00116k_{21} = 0.0000933 + 0.0000666$$

$$k_{21} = 0.0185\ min^{-1}$$

$$k_{10} = \frac{\alpha\beta}{k_{21}} = 0.0386\ min^{-1}$$

$$k_{12} = \alpha + \beta - k_{10} - k_{21} = 0.0421\ min^{-1}$$

Substituting the value for k_{21} into 16 then:

$$V_c = \frac{0.1287-(1.6026 \times 0.0185)}{0.00659} = \frac{0.09905}{0.00659}$$

V_c = 15.0 l

For the corrected intercept values A and B:

$$A = \frac{X_o(\alpha - k_{21})}{V_c(\alpha - \beta)} \qquad B = \frac{X_o(k_{21} - \beta)}{V_c(\alpha - \beta)}$$

$$A = \frac{208(0.0803 - 0.0185)}{15(0.0803 - 0.0089)} \qquad B = \frac{208(0.0185 - 0.0089)}{15(0.0803 - 0.0089)}$$

$$A = \frac{12.85}{1.07} = 12.00 \text{ µg ml}^{-1} \qquad B = \frac{2.00}{1.07} = 1.87 \text{ µg ml}^{-1}$$

$$AUC_C = \frac{A}{\alpha} + \frac{B}{\beta} = \frac{12.00}{0.0803} + \frac{1.87}{0.0089} = 360 \text{ µg ml}^{-1}.\text{min}$$

As mentioned previously, the right atrial blood samples of dogs 1, 2 and 3 have not been considered as mixed venous blood, so the comparison of the pharmacokinetic variables of prilocaine in mixed venous and arterial blood has been restricted to the results obtained from dogs 4, 5 and 6 (Table 4-6). The complete pharmacokinetic data from all six experiments can be found in Appendix 15.

The calculated values for area under the curve (AUC_C) showed some difference from the measured values (AUC_M) with the AUC_C values for both arterial and mixed venous blood samples consistently less than the corresponding AUC_M values. Nevertheless, a two compartment open model and the derivation of the A and B values appear to fit the data sufficiently well.

TABLE 4-6

PHARMACOKINETIC VARIABLES OF PRILOCAINE FOR DOGS 5, 6 AND 7
(mean data ± SD)

	Mixed Venous		Arterial	
	$\bar{x}$	SD	$\bar{x}$	SD
α (min^{-1})	0.081	0.003	0.089	0.008
β (min^{-1})	0.0081	0.0013	0.0086	0.0009
$t_{½\alpha}$ (min)	8.5	0.3	7.9	0.7
$t_{½\beta}$ (min)	88	15	81	9
A ($\mu g\ ml^{-1}$)	13.91	1.61	16.61	3.34
B ($\mu g\ ml^{-1}$)	1.72	0.56	1.86	0.56
AUC_C ($\mu g\ ml^{-1}.min$)	381	46	406	78
AUC_M ($\mu g\ ml^{-1}.min$)	459	85	457	21
Cl_C ($ml\ min^{-1}\ kg^{-1}$)	43.9	6.1	42.0	3.4
Cl_M ($ml\ min^{-1}\ kg^{-1}$)	36.6	3.8	36.8	3.9
Cl_{ss} ($ml\ min^{-1}\ kg^{-1}$)	42.7	4.0	43.6	2.9
V_B ($l\ kg^{-1}$)	4.6	0.9	4.4	0.7
V_c ($l\ kg^{-1}$)	1.1	0.3	0.9	0.2
V_p ($l\ kg^{-1}$)	3.6	1.1	3.4	0.8
k_{10} (min^{-1})	0.040	0.006	0.046	0.009
k_{21} (min^{-1})	0.016	0.004	0.017	0.003
k_{12} (min^{-1})	0.032	0.001	0.034	0.002

Individual data are given in Appendix 15

To further check the validity of the analysis, a computer predicted drug concentration/time profile was created and the data generated subjected to the same analysis as the experimental data.

The program used was SIMULA (in Fortran 4), run on a Burrough's 6900 computer, with a capability of handling up to a three compartment model and several different infusion rates.

The input data required for the computer program were values of V_c, k_{21}, k_{12} and k_{10}. These values were chosen to be in the same order as the previously calculated experimental data. The two infusion rates were the same as used in the experiment, ie $k_o = 4$ mg min^{-1} and $k_o' = 1.6$ mg min^{-1}.

The computer predicted drug concentration data (Fig 4-3) was found to be very similar to the experimental prilocaine concentration data. This data was subjected to pharmacokinetic analysis on the basis of a two compartment open model under the same procedure as used for the experimental data. A comparison of the data fed into the computer program and the data obtained from the pharmacokinetic analysis of the predicted prilocaine concentration data is shown in Table 4-7. The close agreement to these data and the similarity between the AUC_C and AUC_M values confirms the validity of the formula for the derivation of the corrected A and B intercept values for an infusion given at two different rates.

FIGURE 4-3

SEMILOGARITHMIC PLOT OF SIMULA PREDICTED PRILOCAINE CONCENTRATION DATA .v. TIME FOR AN INFUSION GIVEN AT THE TWO RATES USED IN THE DOG EXPERIMENTS

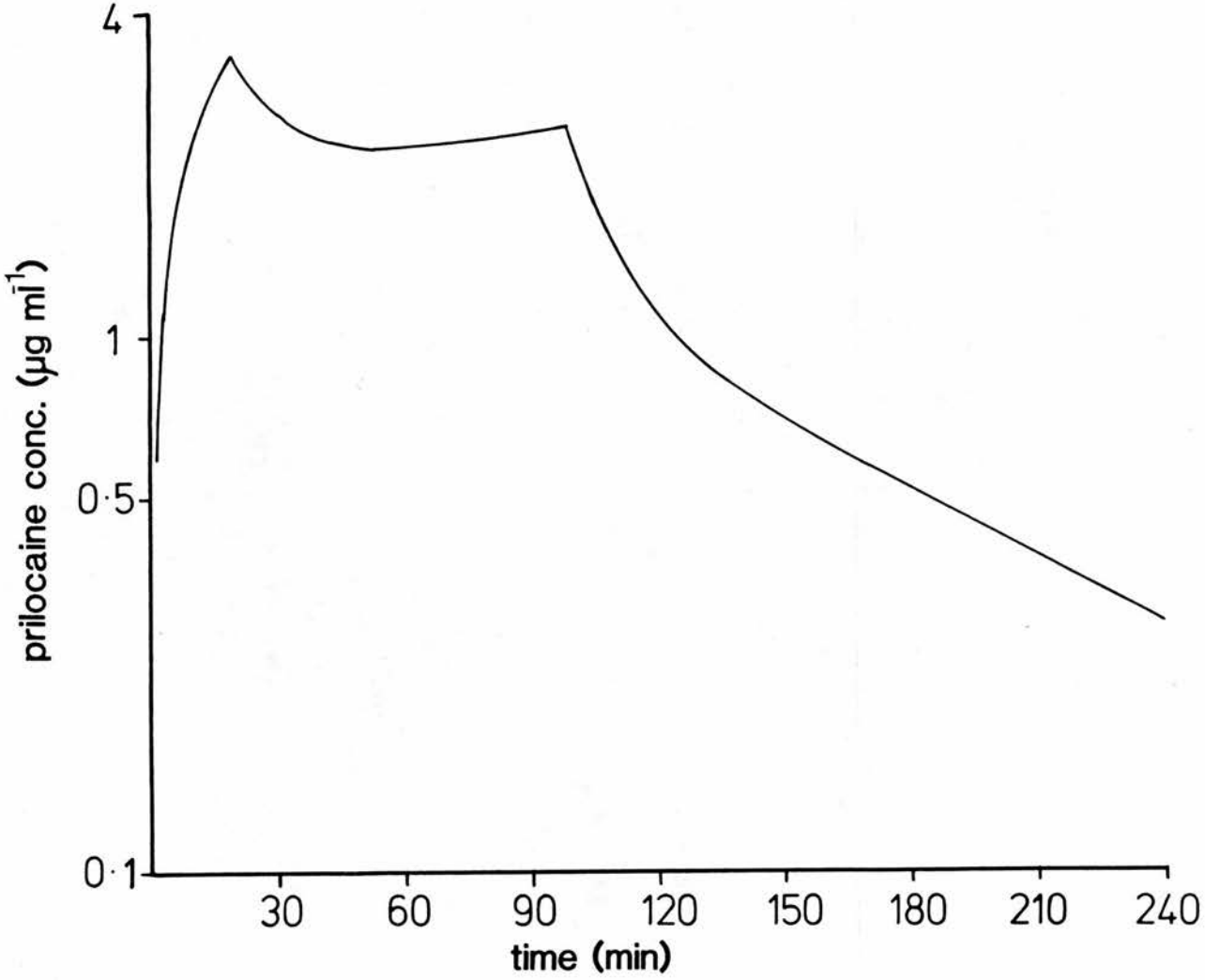


TABLE 4-7

A COMPARISON OF THE PHARMACOKINETIC DATA USED IN THE SIMULA PROGRAM TO PRODUCE THE PREDICTED PRILOCAINE CONCENTRATION DATA AND THE PHARMACOKINETIC DATA CALCULATED FROM THIS CONCENTRATION DATA

	Computer Data	Calculated Data
k_{21} (min^{-1})	0.018	0.019
k_{10} (min^{-1})	0.044	0.049
k_{12} (min^{-1})	0.032	0.037
V_c (l)	12.8	11.4
α (min^{-1})	0.084	0.095
β (min^{-1})	0.0095	0.0099
AUC_C ($\mu g\ ml^{-1}.min$)		372
AUC_M ($\mu g\ ml^{-1}.min$)		362
A ($\mu g\ ml^{-1}$)		16.21
B ($\mu g\ ml^{-1}$)		1.99

As can be seen from Table 4-6, the results of pharmacokinetic analysis of both arterial and venous blood samples are very similar and comparable to the human pharmacokinetic data discussed earlier (Table 1-2). The half life of elimination values of 88 min (mixed venous) and 81 min (arterial) were shorter than the value of 97 min obtained for human volunteers. The volumes of distribution obtained were 4.6 l kg^{-1} (mixed venous) and 4.4 l kg^{-1} (arterial) and these values were comparable to the values of 4.9 l kg^{-1} in humans, with the volumes similarly divided between the central and peripheral compartments in all cases.

As mentioned previously, there is some difference between the AUC_M and AUC_C values, hence the differences between the Cl_C and Cl_M values. Cl_{ss} was calculated using the formula $Cl_{ss} = \frac{k_o}{C_{ss}}$ using the mean C_{ss} values from 60-100 min. The resulting Cl_{ss} values were overestimated since steady state had not been fully established during this 60-100 min time period. The values that will be discussed here are the Cl_M values of 36.6 ml min^{-1} kg^{-1} (mixed venous) and 36.8 ml min^{-1} kg^{-1} (arterial). When compared with the human data of 35.5 ml min^{-1} kg^{-1} it would appear that there was a close agreement of data. However, reported values for hepatic blood flow in various species of anaesthetised dogs vary from 31 ml min^{-1} kg^{-1} (Greenway & Stark, 1971) to 44 ml min^{-1} kg^{-1} (Teramoto & Schumacher, 1962). Ketterer et al (1960)

using a similar anaesthetic technique as used in this study reported the hepatic blood flow of the dog as 38 ml min^{-1} kg^{-1}. When compared to the liver blood flow in man of only 24 ml min^{-1} kg^{-1} (from Price et al, 1960; and the mean weight of the volunteers in the human study of 70 kg) it is apparent that although the clearance of prilocaine in human volunteers is much greater than liver blood flow with a ratio $\frac{\text{clearance}}{\text{hepatic blood flow}}$ of 1.5, this is not the case in the dog, where the ratio $\frac{\text{clearance}}{\text{hepatic blood flow}}$ was 1. As previously stated, all animals were acidotic throughout the experiments but fitting the pH and pCO_2 data to the nomogram produced by Carson et al (1965) showed that cardiac output would have been maintained at normal levels (± 10%). The animals had also experienced some surgical trauma with the insertion of the catheters and this factor was likely to have reduced the hepatic blood flow. It is unlikely however that the hepatic extraction of prilocaine would be 100% and hence it is possible that there is extra-hepatic metabolism in the dog. Tucker et al (1977) showed that hepatic blood flow in human volunteers increased during prolonged infusion of lignocaine, etidocaine and bupivacaine therefore it is possible that this tendency to increase hepatic blood flow would be counteracted by the effect of trauma tending to decrease the flow.

HEPATIC EXTRACTION OF PRILOCAINE IN ANAESTHETISED DOGS

INTRODUCTION

The previous dog experiments had shown that the total body clearance of prilocaine was equal to hepatic blood flow with little or no indication of pulmonary metabolism occurring. Thus, if hepatic extraction of prilocaine in the dog was 100% then liver metabolism of this drug could account for the total body clearance. Hepatic extraction data for the other amide type local anaesthetics in humans (Tucker et al, 1977) had shown that none of these drugs had an extraction value of greater than 75% (etidocaine 73%, lignocaine 68% and bupivacaine 37%).

This experiment was designed to determine the hepatic extraction of prilocaine in the anaesthetised dog and to determine what proportion of the total body clearance was due to extra-hepatic metabolism.

MATERIALS AND METHODS

The Animals and Anaesthesia

Three mongrel dogs (Centre for Laboratory Animals, Bush Estate, Edinburgh, GB) in the weight range 14-17 kg were used in this experiment. Having been fasted overnight, the animals were premedicated with atropine (0.6 mg), given intramuscularly (approximately 1 hr

before the induction of anaesthesia), and then anaesthetised with pentobarbitone sodium given intravenously (25 mg kg^{-1}). Endotracheal intubation was performed and anaesthesia maintained by an infusion of pentobarbitone sodium (4 mg kg^{-1} hr^{-1}) into the left femoral vein. The animals were ventilated (Harvard Animal Respirator, Harvard Apparatus, Millis, Mass, USA) with air, at a rate previously determined to maintain normal blood gas status.

The Preparation

As stated above, the left femoral vein was catheterised for the infusion of pentobarbitone sodium to maintain anaesthesia. The left femoral artery was catheterised to monitor blood pressure, using a Sanborn pressure transducer (Hewlett Packard Ltd, South Queensferry, West Lothian, GB) and a calibrated chart recording of arterial waveform, as well as the ECG, were made. The right femoral artery was catheterised for the sampling of arterial blood, then a radio-opaque catheter inserted into the right femoral vein for later positioning in the hepatic vein.

To catheterise the portal vein and to position the hepatic venous catheter, the abdomen was opened by a midline incision extending from the bottom of the sternum for approximately 40 cm. A tributary of the portal vein was identified and a radio-opaque catheter passed through the tributary and into the portal vein. The catheter was

tied firmly in position, with the tip of the catheter located at a point before the vein merged with the liver tissue. With the abdomen open, the radio-opaque catheter in the right femoral vein was positioned in the hepatic vein using an image intensifier to locate the tip of the catheter.

The abdomen was closed and sutured and a vein in the left forepaw of the animal (left cephalic vein) was cannulated (Medicut, 18-G, Sherwood Medical Industries, Ballymoney, Co Antrim, N Ireland) for the infusion of prilocaine hydrochloride.

All catheters, unless otherwise stated, were Portex, 1.2 mm internal diameter, polyethylene catheters (Portex Ltd, Hythe, Kent, GB). The positioning of the catheters was checked by autopsy at the end of each experiment.

The Infusion

All three dogs received a 15 min infusion of prilocaine hydrochloride. The infusion dose was 10 mg kg^{-1} prepared from 1% Citanest (Astra Chemicals Ltd, Watford, GB) diluted in Hartmann's Solution (Compound Sodium Lactate BP, Travenol Laboratories Ltd, Thetford, Norfolk, GB) to obtain the correct dose in 30 ml. The infusion was given into a vein of the left forepaw at a rate of 2 ml min^{-1} using a Sage syringe infusion pump (Model 355, Orion Research Inc, Cambridge, Mass, USA).

The Samples

Blood samples (3 ml) were withdrawn simultaneously from the femoral artery, the hepatic vein and the portal vein at: 0, 5, 10, 15, 21, 30, 40, 70, 120 and 180 min. Additional samples were withdrawn from the portal vein at 17, 19, 23, 25, 55, 90, 150, 210 and 240 min. The samples were transferred to 10 ml plastic lithium heparin tubes (Brunswick, Sherwood Medical Industries) and stored frozen until assayed for the whole blood prilocaine hydrochloride content using a gas liquid chromatographic technique as described in Appendix 1.

Blood loss was replaced with heparinised Hartmann's Solution (10 units heparin ml^{-1}) which also served to keep the catheters patent.

The whole blood prilocaine concentration data obtained from the portal venous samples was subjected to pharmacokinetic analysis on the basis of a two compartment open model as described in Section 1. The drug concentration data from all the sampling sites was combined to determine the hepatic extraction of prilocaine by the dog.

RESULTS AND DISCUSSION

Heart Rate and Blood Pressure (Appendix 16)

There was a mean decrease in both systolic and diastolic blood pressure of 17 mm Hg over the period of the infusion from initial values of 181 and 132 mm Hg

respectively. This downward trend continued to the end of the experiment at which time the systolic pressure had decreased by 35 mm Hg and the diastolic pressure by 27 mm Hg.

The heart rate remained fairly constant throughout the infusion period in all dogs. By the end of the experiment there had been a mean reduction in heart rate of 12 beat min^{-1} from the initial rate of 159 beat min^{-1}.

Prilocaine Concentration Data

At all sampling times during the infusion, the arterial concentrations were greater than the portal venous concentrations. The mean peak concentrations obtained from all three sampling sites at 15 min were 13.46 μg ml^{-1} (arterial), 9.92 μg ml^{-1} (portal venous) and 4.78 μg ml^{-1} (hepatic venous) (Table 4-8). After terminating the infusion, prilocaine concentrations fell rapidly at all sampling sites (Fig 4-4) with the arterial concentrations generally less than the portal venous concentrations. There then followed a slower decrease in concentrations until the end of the experiment. Hepatic venous prilocaine concentrations were less than both arterial and portal venous concentrations at all times, both during and after the infusion, and with dog 10 no prilocaine could be detected in the 180 min hepatic venous blood sample.

TABLE 4-8

WHOLE BLOOD PRILOCAINE HYDROCHLORIDE CONCENTRATION DATA ($\mu g\ ml^{-1}$) IN PORTAL VENOUS (PV), HEPATIC VENOUS (HV) AND FEMORAL ARTERIAL (A) SAMPLES

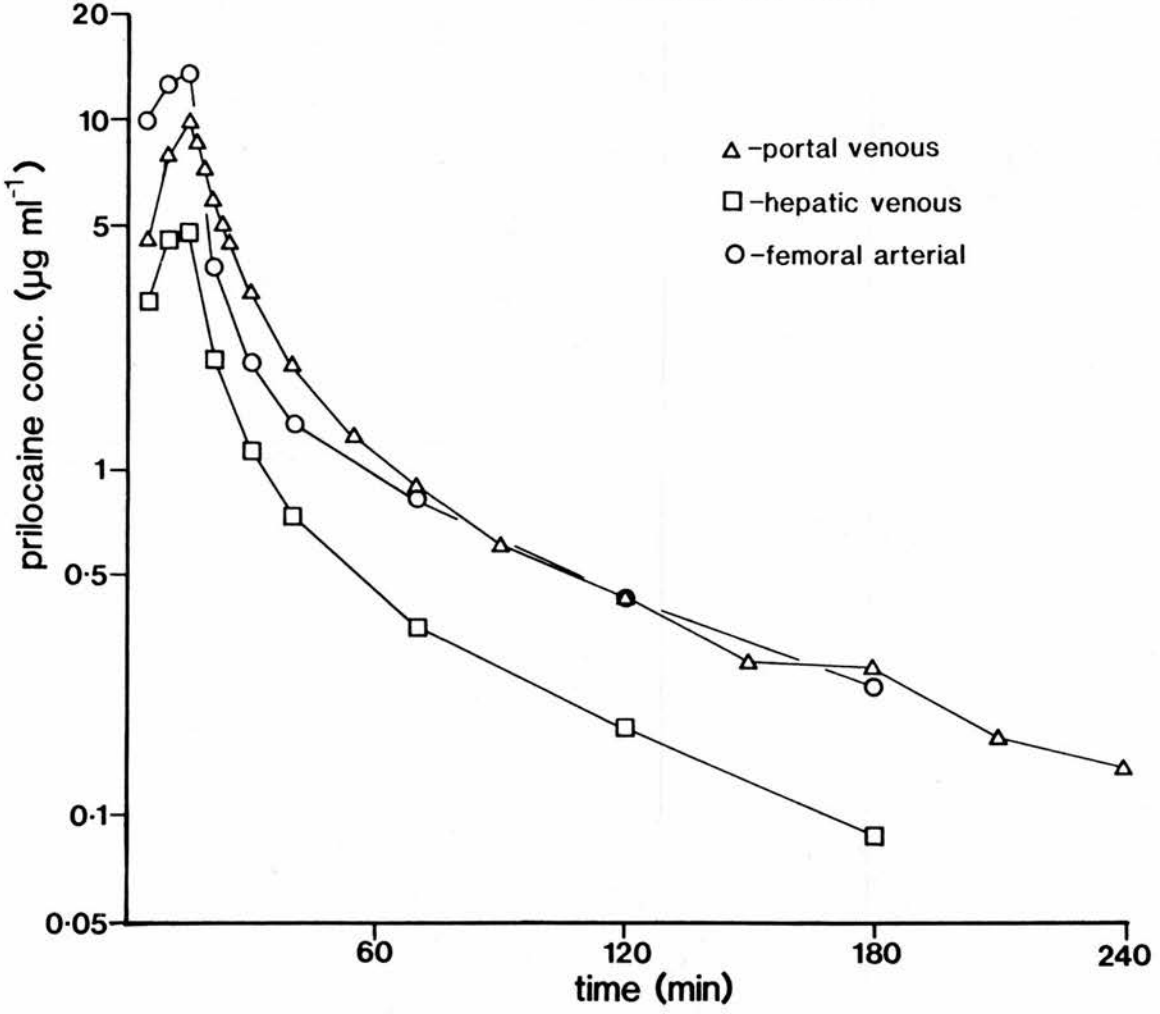
(mean data ± SD)

TIME (min)	PV		HV		A	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
5	4.52	0.21	3.09	2.28	9.90	3.04
10	7.87	1.34	4.56	2.73	12.60	2.76
15	9.92	1.82	4.78	3.07	13.56	1.72
17	8.56	2.00				
19	7.26	1.70				
21	5.91	1.72	2.06	1.08	3.82	0.57
23	5.02	1.38				
25	4.47	1.16				
30	3.22	0.81	1.13	0.61	2.02	0.43
40	1.97	0.36	0.74	0.41	1.36	0.25
55	1.24	0.21				
70	0.89	0.13	0.35	0.22	0.82	0.20
90	0.61	0.17				
120	0.42	0.14	0.18	0.09	0.43	0.14
150	0.28	0.08				
180	0.27	0.07	0.09	0.08	0.24	0.13
210	0.17	0.06				
240	0.14	0.05				

Individual data are given in Appendix 17

FIGURE 4-4

SEMILOGARITHMIC PLOT OF MEAN PRILOCAINE BLOOD CONCENTRATION DATA FROM PORTAL VENOUS, HEPATIC VENOUS AND FEMORAL ARTERIAL SAMPLES .v. TIME



Pharmacokinetic Data

The results obtained from the analysis of the portal venous prilocaine concentration data (Table 4-9) were similar to the data of the previous dog study (Table 4-6). In this study, the AUC_C and AUC_M values were very similar suggesting that the other calculated data were correct. The half life of elimination of 71 min was somewhat shorter than the previously obtained value of 88 min, but the clearance values (Cl_M) of 33.3 ml min^{-1} and 36.6 ml min^{-1} were very similar. The total volume of distribution (V_B) of 3.5 l kg^{-1} was somewhat smaller than the previously obtained value of 4.6 l kg^{-1} but the V_c values in both experiments were very similar, ie 0.9 l kg^{-1} (this experiment) and 1.1 l kg^{-1} (previous experiment). It is apparent that this difference in V_B was due to a difference in the volume of distribution of the peripheral compartment (V_p).

The small differences seen here could however have been due to alterations in the haemodynamic balance of the dogs caused by the relatively extensive surgical procedures required to insert the hepatic catheters.

Hepatic Extraction of Prilocaine

The percentage hepatic extraction was determined from the equation:

$$E = (1 - \frac{HV}{\frac{2}{3}PV + \frac{1}{3}HA}) \times 100$$

TABLE 4-9

PHARMACOKINETIC VARIABLES OF PRILOCAINE FROM PORTAL VENOUS SAMPLES OF DOGS 8, 9 AND 10

(mean data ± SD)

	$\bar{x}$	SD
α (min^{-1})	0.069	0.008
β (min^{-1})	0.0098	0.0006
$t_{½\alpha}$ (min)	10.1	1.1
$t_{½\beta}$ (min)	71	4
AUC_C ($\mu g\ ml^{-1}.min$)	300	72
AUC_M ($\mu g\ ml^{-1}.min$)	313	65
Cl ($ml\ min^{-1}\ kg^{-1}$)	33.3	6.2
V_B ($l\ kg^{-1}$)	3.5	0.7
V_c ($l\ kg^{-1}$)	0.9	0.3
V_p ($l\ kg^{-1}$)	2.7	0.4
k_{10} (min^{-1})	0.043	0.006
k_{21} (min^{-1})	0.016	0.001
k_{12} (min^{-1})	0.020	0.005

Individual data are given in Appendix 18

where E = percentage hepatic extraction

HV = drug concentration in hepatic venous sample

PV = drug concentration in portal venous sample

and HA = drug concentration in hepatic arterial sample (in this experiment, femoral arterial sample)

The division of the input drug concentration into $\frac{2}{3}PV + \frac{1}{3}HA$ is derived from data in Spector (1974) for the relative supply of blood to the liver by the portal vein and hepatic artery in thiopentone sodium anaesthetised dogs.

The result of this analysis for all three preparations are shown in Table 4-10. From this it can be seen that there was very little variation throughout the experimental period within each preparation but there was marked interpreparation variability. The overall mean hepatic extraction value of 59% for prilocaine is less than the values of 73% and 68% for etidocaine and lignocaine respectively, reported by Tucker et al (1977) in human volunteers. The reverse applies in the case of bupivacaine which has been reported as having a hepatic extraction of 55% in the dog (Irestedt et al, 1976) as opposed to only 37% in humans (Tucker et al, 1977).

TABLE 4-10

HEPATIC EXTRACTION OF PRILOCAINE IN DOGS

$$E = \left(1 - \frac{HV}{\frac{2}{3}PV + \frac{1}{3}HA}\right) \times 100$$

DOG	Extraction (%) 8	9	10	$\bar{x}$	SD
TIME (min)					
5	47	23	90	53	34
10	48	33	81	54	25
15	58	38	85	60	24
21	65	37	76	59	20
30	67	41	76	61	18
40	59	38	80	59	21
70	55	41	87	61	24
120	61	27	86	58	30
180	61	30	100	64	35
$\bar{x}$	58	34	85	59	26
SD	7	6	8	3	

E = % hepatic extraction

HV = drug concentration in hepatic venous sample

PV = drug concentration in portal venous sample

HA = drug concentration in hepatic arterial sample (in this experiment, femoral arterial sample)

Using the hepatic blood flow of the dog reported by Ketterer et al (1960) of 38 ml min^{-1} kg^{-1} and the mean hepatic extraction value of prilocaine determined here (59%), the clearance of prilocaine by the dog liver was 22 ml min^{-1} kg^{-1}. The mean total clearance of prilocaine by these three preparations was 33 ml min^{-1} kg^{-1} implying that 11 ml min^{-1} kg^{-1} (33%) of the total clearance of prilocaine was extra-hepatic. From the results of the previous dog experiments it seems unlikely that the lungs could be responsible for this percentage of extra-hepatic metabolism so it is probable that there is another site of extra-hepatic prilocaine metabolism in the dog.

S E C T I O N 5

PHARMACOKINETICS OF PRILOCAINE IN NORMAL AND ANEPHRIC ANAESTHETISED RABBITS

PHARMACOKINETICS OF PRILOCAINE IN ANAESTHETISED RABBITS

INTRODUCTION

The experiments with anaesthetised dogs had indicated that although extra-hepatic metabolism of prilocaine occurred there was no evidence of lung metabolism of this drug.

Kidney slice incubations have been shown to metabolise prilocaine (Geddes, 1965; Åkerman et al, 1966a; and this work, Section 3). The species chosen to investigate the possible renal metabolism of prilocaine was the rabbit and this study was designed to determine the pharmacokinetics of prilocaine in the normal anaesthetised rabbit prior to the investigation of anephric preparations.

MATERIALS AND METHODS

The Animals and Anaesthesia

Three New Zealand White rabbits (mean weight 4.0 kg) were used in this experiment. The animals were placed in a restraining cage and an indwelling needle (Butterfly, 23-G, Abbot Laboratories Ltd, Queensborough, Kent, GB) introduced into a vein in the dorsum of an ear through which a 25% aqueous urethane solution (BDH Chemicals Ltd, Poole, Dorset, GB) was injected slowly until complete anaesthesia was obtained. Further doses of 25% urethane

were administered throughout the experiment to maintain anaesthesia (total mean dose = 10.1 ml kg^{-1}).

The Preparation

A tracheotomy was performed and a Portex T-adaptor (Portex Ltd, Hythe, Kent, GB) used as a tracheotomy tube. The right jugular vein was dissected and a cannula (Medicut, 21-G, Sherwood Medical Industries, Ballymoney, Co Antrim, N Ireland) introduced into the vessel for the infusion of prilocaine hydrochloride. The left jugular vein and left carotid artery were also exposed and similar cannulae introduced for the withdrawal of venous and arterial blood samples. The cannulae were flushed with heparinised 6% dextran (Molecular weight 70,000, Pharmacia AB, Uppsala, Sweden) to maintain patency.

The Infusion

The prilocaine hydrochloride infusion of 10 mg kg^{-1} (prepared from Citanest, 0.5%, Astra Chemicals Ltd, Watford, GB, diluted with saline to the appropriate concentration) was given over a period of 15 min in a volume of 7.5 ml using a Harvard syringe infusion pump (Harvard Apparatus, Millis, Mass, USA) at a rate of 0.5 ml min^{-1}.

The Samples

Blood samples (0.7 ml) were withdrawn from the left jugular vein and left carotid artery simultaneously

at the following times: 0, 5, 10, 15, 17, 19, 21, 25, 30, 40, 55, 70, 100, 130, 160, 190, 220 and 250 min. These samples were stored in 1 ml EDTA tubes under refrigeration until assayed for whole blood prilocaine hydrochloride concentrations using the gas liquid chromatographic technique described in Appendix 1.

The prilocaine concentration data was then subjected to pharmacokinetic analysis on the basis of a two compartment open model as described previously (Section 1).

RESULTS AND DISCUSSION

Prilocaine Hydrochloride Concentration Data (Appendix 19)

During the infusion period, the arterial prilocaine concentrations were much greater than the venous concentrations as would be expected. The mean peak concentration for arterial samples was 6.26 $\mu g\ ml^{-1}$ at 15 min and for venous samples at the same time interval 3.36 $\mu g\ ml^{-1}$. After the end of the infusion the arterial prilocaine concentrations fell more rapidly than the venous concentrations but in two of the three preparations (nos 1 and 3; Figure 5-1) there was a temporary increase in the arterial concentrations 25 min and 10 min respectively after the end of the infusion.

Pharmacokinetic Analysis (Table 5-1)

The characteristics of the arterial prilocaine concentration data of the rapid redistribution phase of

FIGURE 5-1

INDIVIDUAL SEMILOGARITHMIC PLOTS OF PRILOCAINE BLOOD CONCENTRATIONS IN NORMAL ANAESTHETISED RABBITS AFTER INTRAVENOUS INFUSION .v. TIME

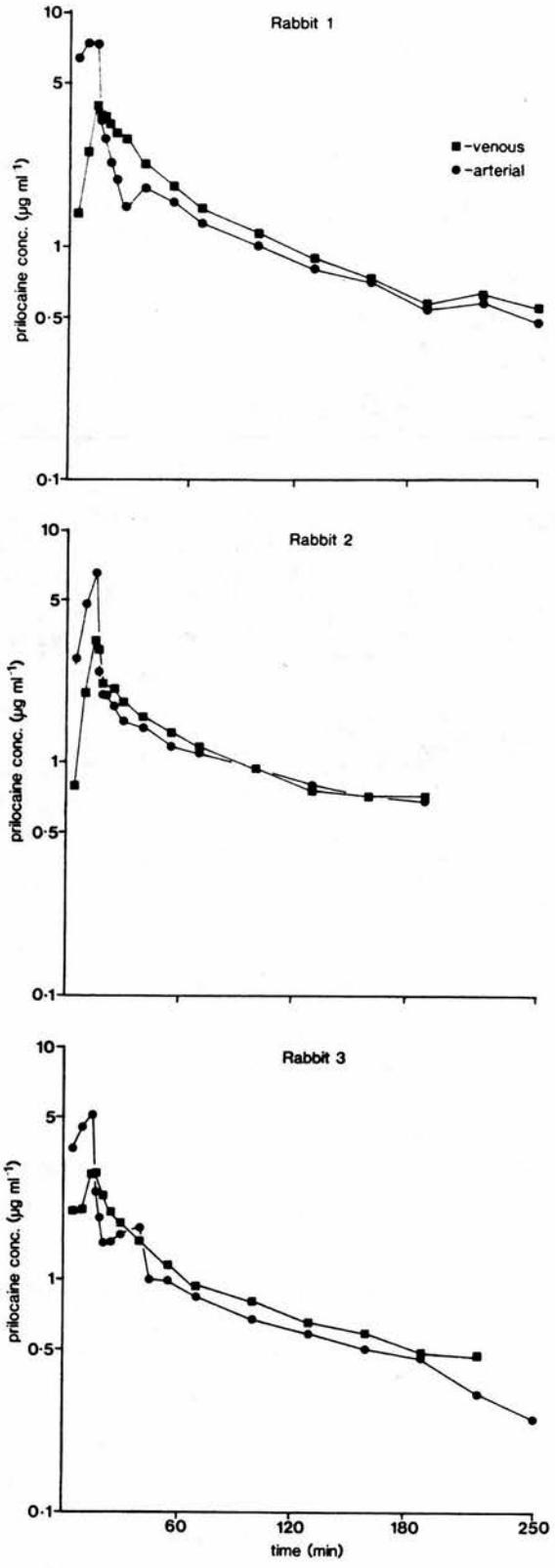


TABLE 5-1

PHARMACOKINETIC VARIABLES OF PRILOCAINE IN RABBITS

(mean data ± SD)

	Venous		Arterial	
	$\bar{x}$	SD	$\bar{x}$	SD
α (min^{-1})	0.056	0.002	0.173	0.143
β (min^{-1})	0.0055	0.0019	0.0054	0.0013
$t_{½\alpha}$ (min)	12.4	0.4	5.9	3.5
$t_{½\beta}$ (min)	134	40	134	32
A ($\mu g\ ml^{-1}$)	2.41	0.18	10.99	14.64
B ($\mu g\ ml^{-1}$)	1.64	0.59	1.44	0.17
AUC_C ($\mu g\ ml^{-1}.min$)	340	40	324	89
AUC_M ($\mu g\ ml^{-1}.min$)	345	40	366	68
Cl ($ml\ min^{-1}\ kg^{-1}$)	28.2	2.6	27.0	5.3
V_B ($l\ kg^{-1}$)	5.6	1.8	5.9	1.2
V_c ($l\ kg^{-1}$)	2.4	0.5	1.8	1.3
V_p ($l\ kg^{-1}$)	3.1	1.3	4.1	0.4
k_{10} (min^{-1})	0.012	0.002	0.034	0.036
k_{21} (min^{-1})	0.025	0.004	0.031	0.006
k_{12} (min^{-1})	0.024	0.005	0.112	0.114

Individual data are given in Appendix 20

the drug, particularly in the first preparation, resulted in quite large deviations in some of the calculated arterial pharmacokinetic variables. However, a comparison of the arterial and venous mean pharmacokinetic data showed them to be quite similar with little difference between the AUC_M values and, as a result of this, the clearance values of 27.0 ml min^{-1} kg^{-1} (arterial) and 28.2 ml min^{-1} kg^{-1} (venous) were very similar. These clearance values are much lower than the values reported previously for unanaesthetised human volunteers (35.5 ml min^{-1} kg^{-1}) and anaesthetised dogs (36.6 ml min^{-1} kg^{-1}). However, the liver blood flow of unanaesthetised rabbits has been reported as 37 ml min^{-1} kg^{-1} (White et al, 1967) and Wyler (1974) has reported that in chlorolase-urethane anaesthetised rabbits, liver blood flow is only 76% that of unanaesthetised rabbits. Although these rabbits were anaesthetised with urethane only, it is probable that the hepatic blood flow could be about 28 ml min^{-1} kg^{-1}. This would mean that, as in the anaesthetised dog, clearance of prilocaine in the anaesthetised rabbit is virtually the same as hepatic blood flow. As it is unlikely that the hepatic extraction of prilocaine in the rabbit is 100% it would appear that there is some degree of extrahepatic metabolism of prilocaine.

The mean values for the half life of elimination at both sampling sites were identical (134 min). This value exceeded the values from both the human volunteer

(97 min) and dog studies (88 min). The main differences between the pharmacokinetic variables obtained from venous and arterial prilocaine concentration data were the α values and hence the $t_{\frac{1}{2}\alpha}$ values and also the k_{12} values, this being due to the apparently very rapid redistribution phase observed in the arterial concentration data. The large difference between the values for the A intercepts is due to a very high A value in one preparation only.

The mean V_c values differed between the two sampling sites, being 2.4 l kg^{-1} from venous samples and 1.8 l kg^{-1} from arterial samples. Both these values were greater than the values for V_c found in either humans (1.3 l kg^{-1}) or dogs (1.1 l kg^{-1} mixed venous and 0.9 l kg^{-1} arterial).

As the clearance data from these experiments, and the tissue incubation data reported earlier, suggest extra-hepatic prilocaine metabolism in the rabbit, it was decided to examine further the fate of prilocaine in the anaesthetised rabbit.

PHARMACOKINETICS OF PRILOCAINE IN ANAESTHETISED ANEPHRIC RABBITS

INTRODUCTION

The results obtained from the previous rabbit experiments had shown that the total body clearance of prilocaine was approximately equal to the hepatic blood flow. It was assumed that the hepatic extraction of prilocaine in this preparation was less than 100% as had been found in the dog (Section 4). It was therefore proposed that if the kidneys of the rabbit were metabolising prilocaine, then the total body clearance of prilocaine in anephric preparations would be less than in normal preparations. This experiment was designed to test this hypothesis.

MATERIALS AND METHODS

The Animals and Anaesthesia

Four New Zealand White rabbits were used in this experiment (mean weight = 2.4 kg). The anaesthetic technique was as previously described in the study with normal rabbits except that the mean dose of 25% urethane was 8.1 ml kg^{-1}.

The Preparation

The animals were prepared as described in the preceding experiment with the following exceptions:

(1) there was no cannula in the left carotid artery;
(2) blood samples were taken only from the left jugular vein, at the same time intervals as in the previous experiment.

After the initial preparation, the kidneys were exposed by opening the abdomen with a midline incision and displacement of the intestines. The kidneys were separated from the surrounding connective tissue and a loop of string passed over each kidney. Each loop was pulled tightly over the renal artery and vein to occlude the renal blood circulation. The abdomen was closed and prilocaine hydrochloride infused at 10 mg kg^{-1} (as previously described) for 15 min.

The Samples

0.7 ml blood samples were withdrawn from the left jugular vein at the same time intervals as in the previous experiment. The storage and analysis of the samples were again as previously described.

Pharmacokinetic analysis of the prilocaine blood concentration data was attempted by fitting this data to a two compartment open model as has been previously described in Section 1.

RESULTS AND DISCUSSION

Prilocaine Hydrochloride Concentration Data

The whole blood prilocaine concentration data was not as expected. In both preparations 1 and 2, there appeared to be little redistribution of the drug after the end of the infusion (Fig 5-2) with the concentration of the drug in the blood remaining at relatively high levels, rabbit 2 having a concentration of 4.65 $\mu g\ ml^{-1}$ at 130 min when it died. Rabbit 1 survived for the full experimental period and at 250 min the venous prilocaine concentrations had only fallen to 0.86 $\mu g\ ml^{-1}$ compared with 0.09 and 0.17 $\mu g\ ml^{-1}$ in rabbits 3 and 4 respectively.

Pharmacokinetic Analysis (Table 5-2)

Although there appeared to be little evidence of a redistribution phase in preparations 1 and 2, the estimated half life of elimination values were 100 and 130 min respectively which are in the same order as the $t_{\frac{1}{2}\beta}$ value obtained with the normal rabbits (134 min). Preparations 3 and 4 produced time concentration profiles more typical of a two compartment open model (Fig 5-2) with $t_{\frac{1}{2}\beta}$ values of 68 min and 120 min respectively, the former figure being much shorter than that found in the normal preparations (134 min). The V_B values for these preparations were greater than for normal rabbits, apparently due to an increase of the V_p values, with the V_c values similar to the normal data. The k_{10} values

FIGURE 5-2

INDIVIDUAL SEMILOGARITHMIC PLOTS OF PRILOCAINE BLOOD CONCENTRATIONS IN ANEPHRIC ANAESTHETISED RABBITS AFTER INTRAVENOUS INFUSION .v. TIME

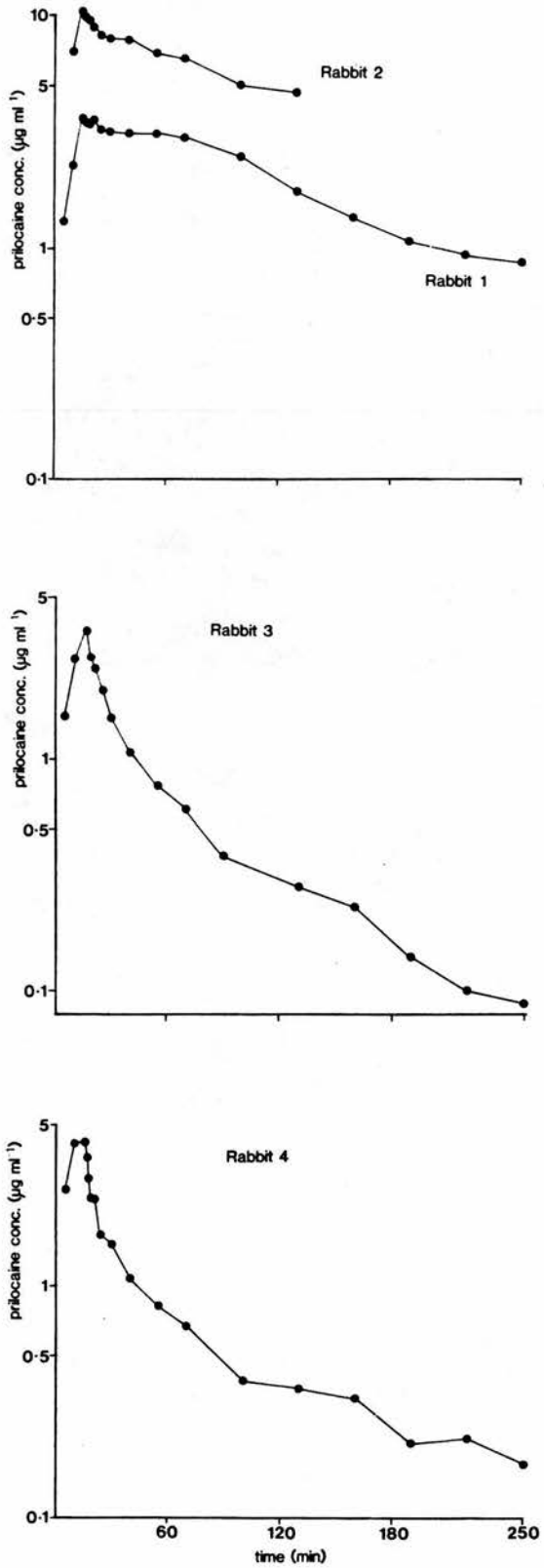


TABLE 5-2

PHARMACOKINETIC VARIABLES OF PRILOCAINE HYDROCHLORIDE IN ANEPHRIC ANAESTHETISED RABBITS

Preparation	3	4
A ($\mu g\ ml^{-1}$)	3.59	3.38
B ($\mu g\ ml^{-1}$)	0.97	0.70
AUC_C ($\mu g\ ml^{-1}.min$)	152	186
AUC_M ($\mu g\ ml^{-1}.min$)	153	205
Cl_C ($ml\ min^{-1}\ kg^{-1}$)	65.8	53.8
Cl_M ($ml\ min^{-1}\ kg^{-1}$)	65.4	48.8
V_B ($l\ kg^{-1}$)	6.4	8.4
V_c ($l\ kg^{-1}$)	2.2	2.5
V_p ($l\ kg^{-1}$)	4.2	5.9
k_{10} (min^{-1})	0.030	0.021
k_{21} (min^{-1})	0.022	0.014
k_{12} (min^{-1})	0.022	0.023
$t_{½\alpha}$ (min)	10.9	13.7
$t_{½\beta}$ (min)	68	120
α (min^{-1})	0.064	0.052
β (min^{-1})	0.0102	0.0058

were higher than the normal values in both preparations (0.030 min^{-1} for 3 and 0.021 min^{-1} for 4, 0.012 min^{-1} for normal) suggesting a more rapid elimination of drug from the body by the anephric preparations than the normal preparations. This fact is confirmed by the very high clearance values of the anephric preparations of 65.4 ml min^{-1} kg^{-1} (3) and 48.8 ml min^{-1} kg^{-1} (4) which are approximately two times greater than the normal value (28.2 ml min^{-1} kg^{-1}).

It would appear from these results that an anephric rabbit preparation is not a suitable technique to use in assessing the role of the kidneys as a site of prilocaine metabolism as this procedure appears to have an adverse effect on the haemodynamic balance of the preparation. In the case of preparations 1 and 2, it is probable that the trauma of anaesthesia and surgery caused a collapse of the circulation, particularly to the peripheral tissues and the viscera and hence greatly reducing the redistribution of the drug. In the case of preparations 3 and 4, it is possible that, with no adverse effects caused by surgical trauma, the loss of the renal circulation would cause 25% of the cardiac output, ie that which had been supplying the renal circulation, to be redistributed to other tissues. The most likely site for redistribution of this increased blood flow would be by dilation of the splanchnic blood vessels, resulting in an increase in the overall hepatic blood flow. This would be reflected by an increase in prilo-

caine clearance. Bromage & Gertel (1972) reported that the duration of brachial plexus blocks in patients with chronic renal failure was shorter than in normal patients. They stated that this could be due to increased cardiac output in renal failure. However, Thompson et al (1971) found no differences in pharmacokinetic variables of lignocaine in normal patients and patients with renal failure. Therefore, to look at possible renal metabolism in the rabbit, an isolated perfused kidney preparation would appear to be a more suitable technique.

S E C T I O N 6

LUNG UPTAKE OF LIGNOCAINE AND PRILOCAINE IN ANAESTHETISED HUMAN SUBJECTS

FIRST PASS EFFECT OF THE HUMAN LUNG ON LIGNOCAINE AND PRILOCAINE BLOOD CONCENTRATIONS

INTRODUCTION

Experiments with isolated perfused rat lungs (Section 2) had shown that lung tissue was capable of accumulating large amounts of both lignocaine and prilocaine at steady state perfusate concentrations. However, to protect the brain from toxic concentrations of local anaesthetics, lung tissue would be required to absorb large amounts of these drugs on their first passage through this organ. In experiments with anaesthetised pigs, it had been shown that after an intravenous bolus injection of lignocaine, the lungs could extract 44% of the administered dose on a single passage through the pulmonary circulation (Bertler et al, 1978). Using a similar technique with unanaesthetised human volunteers, Jorfeldt et al (1979) found a 60% extraction of lignocaine by the lungs.

This experiment was designed to compare the lung uptake of prilocaine and lignocaine in humans on a single passage through this organ after the simultaneous intravenous administration of equal amounts of each drug.

MATERIALS AND METHODS

The protocol for this experiment was passed by the Ethical Committee of the South Lothian District Division of Anaesthesia.

Patients undergoing general anaesthesia for middle ear surgery were studied. Radial artery cannulation (Butterfly, 23-G, Abbot Laboratories, Queensborough,

Kent, GB) for arterial blood pressure monitoring was a routine procedure with these patients and this cannula was used for the withdrawal of blood samples. Another catheter (EZ 19-G, Deseret Pharmaceutical Co Inc, Sandy, Utah, USA) was introduced into the ante-cubital vein of the contralateral arm and advanced until it was judged that the tip of the catheter was in a vein at the level of the shoulder joint.

A mixture of 15 mg lignocaine hydrochloride monohydrate (1.5 ml of 1% Lidothesin, Pharmaceutical Manufacturing Co, Bolton, GB), 15 mg prilocaine hydrochloride (1.5 ml of 1% Citanest, Astra Chemicals Ltd, Watford, GB) and 5 mg indocyanine green (ICG) (Cardio Green, Hynson, Westcott & Dunning, Baltimore, Maryland, USA made up to 2.5 mg ml^{-1}), giving a total volume of 5 ml, was injected through the venous catheter over a period of not more than 2 sec, immediately followed by an injection of 10 ml saline to flush the catheter.

As the injection was made, continuous blood sampling was commenced from the arterial cannula using a peristaltic pump (Harvard Apparatus, Millis, Mass, USA). For the first 90 sec period the rate of blood withdrawal was approximately 20 ml min^{-1} and this was divided into 12 consecutive samples taken over 7.5 sec intervals. For the next 30 sec the sampling rate was reduced to approximately 10 ml min^{-1} and this was divided into two samples taken over 15 sec intervals. The withdrawal rate

was then reduced to approximately 5 ml min^{-1} for a further period of 120 sec and this was divided into two samples taken over 60 sec intervals.

The blood samples were collected into plastic lithium heparin tubes (Brunswick, Sherwood Medical Industries, Ballymoney, Co Antrim, N Ireland) and centrifuged as soon as possible to obtain the plasma. The plasma samples were analysed for ICG content using a spectrophotometric technique and then each plasma sample remixed with its corresponding red cell fraction and the whole blood assayed for lignocaine hydrochloride monohydrate and prilocaine hydrochloride using a gas liquid chromatographic technique (Appendix 1).

The experimental procedure was undertaken twice with each subject. The first injection was made shortly after induction of anaesthesia when it had been predicted that the patient would be acidotic. The second injection was made after the end of the operation and following a period of hyperventilation of the patient which was intended to move the patient's blood gas status towards alkalosis.

Before each experiment, arterial blood samples were withdrawn for blood gas analysis, determination of haematocrit value (for the conversion of plasma ICG concentrations to whole blood concentrations) and to obtain normal plasma with which to calibrate the ICG assay.

Spectrophotometric Determination of ICG in Human Plasma

The instrument used was a Unicam SP 500 Spectrophotometer (Unicam Instruments Ltd, Edinburgh, GB). The absorption of each sample was measured at a wavelength of 805 nm using the null balance technique against a blank plasma sample taken immediately before each experiment. Due to the small volume of the samples, a 2 mm optical path length quartz cell with a total volume of 0.8 ml was used.

Calibration curves were prepared from various samples of normal plasma by spiking 1 ml aliquots of plasma with microlitre quantities of 2.5 mg ml^{-1} ICG solution. Although the relationship between absorption and concentration was found to be linear up to 20 µg ml^{-1}, there were variations in the results obtained from different subjects' plasma samples. As a result of this, a blood sample was taken prior to each experiment and the plasma from this used to perform a calibration curve for the samples obtained from that experiment.

Because ICG is highly bound to plasma protein, of which albumin is the principle carrier (95%), it was necessary to determine the haematocrit value of each subject's blood for each experiment to correct the determined plasma ICG concentration to whole blood ICG concentrations:

$$\text{Whole blood ICG conc} = \frac{\text{Plasma ICG Conc}}{100} \times \% \text{ plasma}$$

RESULTS AND DISCUSSION

The anticipated changes in the blood gas status between the two separate experiments were not as marked as had been expected. During the first run, four of the five subjects studied could be regarded as being in an acidotic state (Table 6-1), but in the second run only two of the subjects had blood gases approaching a state of alkalosis. Hyperventilation reduced the pCO_2 values in all subjects between runs one and two, but an increase in pO_2 values occurred only in two of the five subjects. The mean increase in pH between runs one and two was only 0.10 pH unit.

Initially, all blood concentrations of ICG were multiplied by a factor of three to correct these values for an injection of 15 mg of this drug, equivalent to the dose of each local anaesthetic. The concentrations of drug in the blood were compared by two methods. The peak lignocaine and prilocaine concentrations were expressed as a percentage of the peak ICG concentrations (Table 6-2). In all cases these values were lower for prilocaine than for lignocaine, with overall mean values of 43% and 59% respectively, implying that prilocaine was being retarded by the lungs to a greater extent than lignocaine. With the exception of subject 1, the peak blood concentrations of prilocaine were much lower than those of lignocaine.

TABLE 6-1

BLOOD GAS ANALYSIS OF ARTERIAL BLOOD

Subject	RUN 1			RUN 2		
	pO_2 (kPa)	pCO_2 (kPa)	pH	pO_2 (kPa)	pCO_2 (kPa)	pH
1	26.3	7.6	7.27	43.6	7.1	7.28
2	16.9	9.9	7.18	16.5	8.9	7.23
3	28.0	6.0	7.35	26.6	4.5	7.44
4	21.6	7.5	7.24	32.0	4.2	7.45
5	25.9	8.2	7.20	24.9	4.8	7.36
$\bar{x}$	23.7	7.8	7.25	28.7	5.9	7.35
SD	4.5	1.4	0.67	10.0	2.0	0.97

TABLE 6-2

% OF THE PEAK ICG CONCENTRATIONS OF PEAK PRILOCAINE AND LIGNOCAINE CONCENTRATIONS

$$\frac{\text{Peak Lignocaine Concentration}}{\text{Peak ICG Concentration}} \times 100\ (\%)$$

Subject	1	2	3	4	5	$\bar{x}$	SD	Total data $\bar{x}$	Total data SD
Run 1	70	31	72	61	60	59	16	59	16
Run 2	84	52	72	49	40	59	18		

$$\frac{\text{Peak Prilocaine Concentration}}{\text{Peak ICG Concentration}} \times 100\ (\%)$$

Subject	1	2	3	4	5	$\bar{x}$	SD	Total data $\bar{x}$	Total data SD
Run 1	69	18	52	40	53	46	19	43	18
Run 2	68	16	49	32	35	40	19		

The difference between the data for lignocaine and prilocaine was statistically significant ($p < 0.001$, using Student's paired t test)

Fig 6-1 shows blood drug concentration plotted against time for both runs of one of the patients studied (subject 2) and illustrates well the differences between the three different drug concentrations throughout the sampling period.

The areas under the drug concentration/time curves (AUC) were measured up to the time when recirculation of the ICG occurred, as judged from the shape of the ICG concentration/time profile. Similar to the peak blood concentration data, the AUC values for lignocaine and prilocaine have been expressed as a percentage of the AUC values for ICG (Table 6-3). The AUCs for prilocaine were only 48% (overall mean value) of those for ICG with the AUC values for lignocaine nearly 20% greater at 67% (overall mean value).

As can be seen from both Tables 6-2 and 6-3 there were no remarkable differences between the two experiments with each subject which was not surprising, as there was little change in the overall blood gas status between experiments with any of the subjects studied.

ICG has been utilised as an inert marker by other workers studying lung uptake of drugs. Davis & Wang (1965) demonstrated a 33% removal of C^{14}-5-hydroxy-tryptamine in dogs and Geddes et al (1978) found a 75% first pass uptake of C^{14}-propranolol in man with a method involving the simultaneous injection of ICG and drug. Utilising a method similar to the one used in these

FIGURE 6-1

ICG, LIGNOCAINE AND PRILOCAINE BLOOD CONCENTRATIONS IN SUBJECT 2 AFTER INTRAVENOUS BOLUS INJECTION OF A MIXTURE OF THESE THREE DRUGS
(Run 1 - pH = 7.18; Run 2 - pH = 7.23)

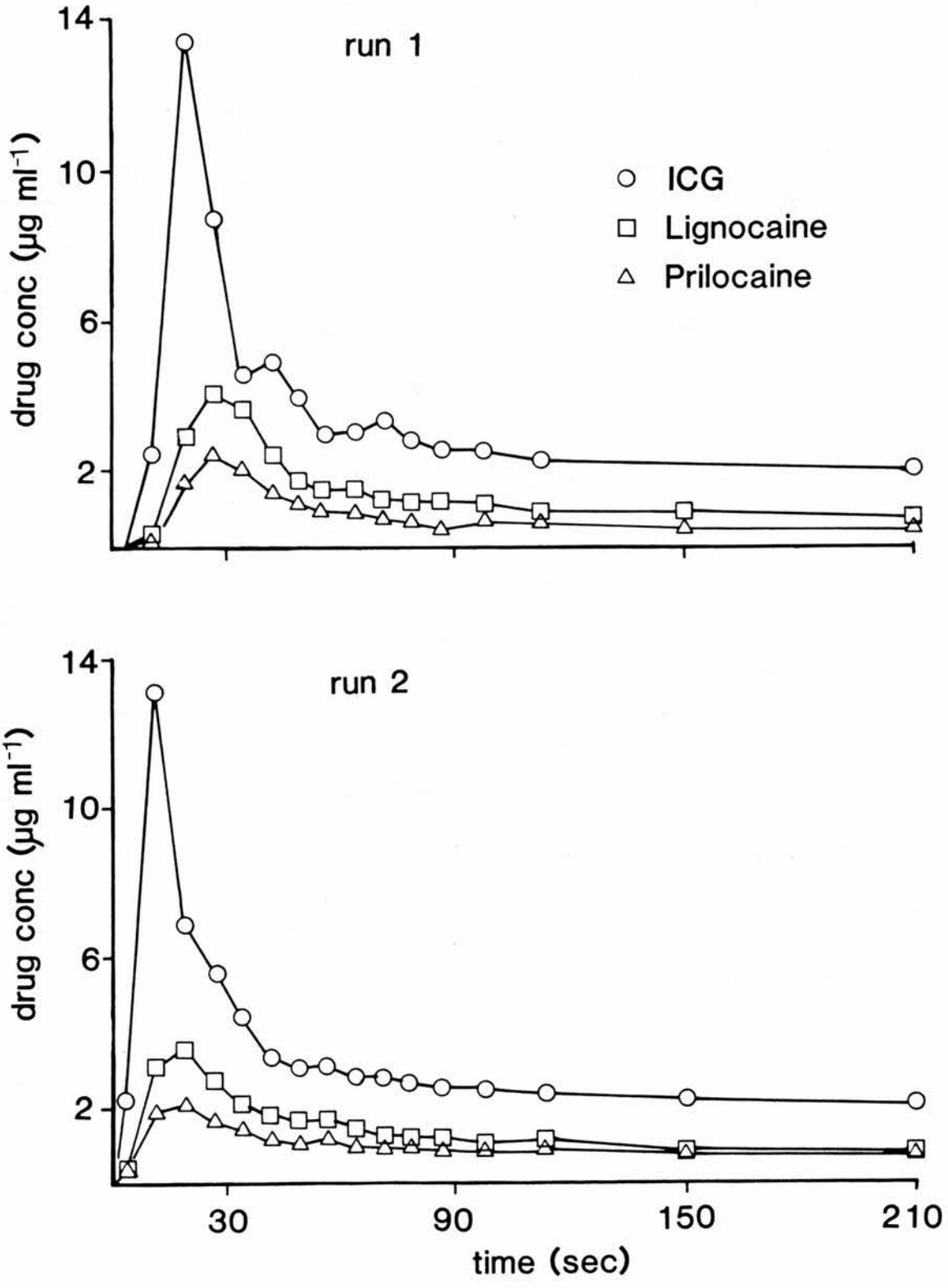


TABLE 6-3

% OF THE $AUC_{(ICG)}$ OF $AUC_{(LIGNOCAINE)}$ AND $AUC_{(PRILOCAINE)}$

$$\frac{AUC_{(LIG)}}{AUC_{(ICG)}} \times 100\ (\%)$$

Subject	1	2	3	4	5	$\bar{x}$	SD	Total data $\bar{x}$	Total data SD
Run 1	85	38	83	74	56	67	20	67	22
Run 2	103	35	83	59	53	67	27		

$$\frac{AUC_{(PRIL)}}{AUC_{(ICG)}} \times 100\ (\%)$$

Subject	1	2	3	4	5	$\bar{x}$	SD	Total data $\bar{x}$	Total data SD
Run 1	76	22	64	47	38	49	21	48	21
Run 2	79	21	58	37	39	47	22		

The difference between the data for lignocaine and prilocaine was statistically significant ($p < 0.001$, using Student's paired t test)

experiments, Bertler et al (1978) showed a 44% first pass uptake of lignocaine in anaesthetised pigs and using the same technique Jorfeldt et al (1979) reported a 60% first pass uptake of lignocaine in unanaesthetised volunteers.

The uptake of lignocaine reported here using the method of AUC comparisons (33%) was less than the value reported by Jorfeldt et al (60%). This may be due to the fact that in this study the subjects were anaesthetised and were receiving other drugs which could have interfered with the pulmonary uptake of the lignocaine. Also, prilocaine was injected simultaneously with the lignocaine and these two drugs were probably competing with each other for the same uptake sites.

The mean reduction in the peak concentrations of 57% for prilocaine and 41% for lignocaine is likely to be a significant factor in reducing the toxic effects of a bolus injection of either of these local anaesthetics on the central nervous system by reducing the tissue/blood drug concentration gradient and hence reducing the amount of drug the central nervous system tissue will absorb. The greater lung absorption of prilocaine than lignocaine in humans observed in this study is in agreement with the data presented earlier for the isolated rat lung preparations.

GENERAL DISCUSSION

GENERAL DISCUSSION

The initial objectives of this study were to describe the pharmacokinetics of prilocaine in man and thereafter study the factors controlling the distribution and clearance of the amide type local anaesthetics.

It is known that very small quantities of the amide type local anaesthetics are excreted unchanged in the urine and that the main site of metabolism of these drugs is in the liver (Tucker et al, 1977). As a result of this, the total body clearance values for these drugs are necessarily less than hepatic blood flow. The results from the prilocaine pharmacokinetic study in humans (Section 1) showed the total body clearance of prilocaine (2.42 l min^{-1}) to exceed the liver blood flow (1.7 l min^{-1}, Price et al, 1960). Published values for the urinary excretion of prilocaine in human volunteer studies range from less than 1% (Mather, 1972 in Mather & Tucker, 1978) to approximately 6% in acidified urine (Boyes, 1967). The higher published figure for the urinary excretion of prilocaine would still leave over 20% of the clearance of prilocaine unaccounted for, assuming the hepatic extraction of prilocaine to be 100%. Therefore, it was proposed that an extrahepatic site of prilocaine metabolism existed.

Although prilocaine is the least lipid soluble of the amide type local anaesthetics (Covino & Vassallo, 1976) it was found to have a large volume of distribution in humans

(343 l). Only etidocaine which is more than 100 times more lipid soluble than prilocaine has a greater volume of distribution (666 l). This large volume of distribution and the high clearance of prilocaine were also found in the dog and rabbit studies (Sections 4 and 5). There was little interspecies variation in the volume of distribution data when expressed in terms of body weight (1 kg^{-1}). Using the values derived from the venous prilocaine concentration data, V_B rabbit (5.6 l kg^{-1}) > V_B human (4.9 l kg^{-1}) > V_B dog (4.6 l kg^{-1}). Similarly, the half lives of elimination decreased in the order, $t_{\frac{1}{2}\beta}$ rabbit (134 min) > $t_{\frac{1}{2}\beta}$ human (97 min) > $t_{\frac{1}{2}\beta}$ dog (88 min). In the dog and rabbit studies, where both arterial and venous blood samples were taken, the differences between the pharmacokinetic variables derived from the prilocaine concentration data of the two sampling sites, were minimal. In the dog and rabbit studies, the clearance of prilocaine was found to be approximately equal to hepatic blood flow rather than exceeding it as had been found in the human study. However, experiments to determine the hepatic extraction of prilocaine in the dog indicated that 33% of the total clearance of this drug was extrahepatic (Section 4).

The greater total body clearance of prilocaine compared to the other amide type local anaesthetics is probably related to the method of its metabolic degradation. It has been shown that for lignocaine which is a tertiary amine, N-dealkylation is the initial metabolic step prior to the hydrolysis of the amide bond (Hollunger, 1960a;

Boyes, 1967), whereas for prilocaine which is a secondary amine, hydrolysis of the amide bond is the initial metabolic reaction to occur (Geddes, 1965; Åkerman et al, 1966a). Prilocaine has been shown to be more rapidly metabolised than lignocaine in vivo (Åkerman et al, 1966a) suggesting that either the amidase responsible for the hydrolysis of prilocaine is more available than the enzyme responsible for the N-dealkylation of lignocaine or, that the hydrolytic reaction is more facile than dealkylation.

Metabolism of prilocaine by lung and kidney slice incubations was reported by Åkerman et al (1966a) but no lignocaine metabolism by these preparations was found. Similarly, Post et al (1978) could not detect lignocaine metabolism in lung slice preparations. The metabolism of prilocaine by dog and rabbit lung and kidney slice incubations has been confirmed (Section 3), but unlike the previous work lung tissue appeared to have some capability for lignocaine metabolism. These results, together with the data obtained from the isolated perfused rat lung preparations (Section 2), which indicated that large amounts of prilocaine were accumulated by lung tissue, led to the investigation of the lung as the site of extrahepatic metabolism. The lung seemed a more attractive choice than the kidneys as the site of extrahepatic metabolism since it receives the total cardiac output, thus requiring a much lower extraction of the drug than would be necessary by the kidneys.

The *in vivo* work with dogs (Section 4) suggested that little or no metabolism of prilocaine occurred in the intact lung. A small significant decrease in steady state blood prilocaine concentrations between pulmonary arterial and aortic samples was found in two of the three preparations studied, but this extraction of prilocaine would not account for the extrahepatic metabolism of this drug. It was shown that steady state blood prilocaine concentrations had not quite been obtained during the infusion and it is possible that the difference in concentrations at the two sampling sites were a result of lung uptake of the drug. Although this does not exclude the possibility of pulmonary metabolism of prilocaine in humans, it does nevertheless make it seem less likely.

To investigate the possibility of renal metabolism of prilocaine, a comparison was made of the clearance values in normal and anephric anaesthetised rabbits (Section 5). The results showed that tying off the renal circulation actually increased the clearance of prilocaine. This was thought to have been caused by the fraction of the cardiac output normally intended for the renal circulation being redirected to the splanchnic circulation, thus increasing the overall hepatic blood flow. This in turn would cause an increase in the clearance of drug by the liver. The possibility of renal metabolism remains to be investigated further. It is unlikely that this could account for the total extrahepatic metabolism of prilocaine in man, as the renal extraction of prilocaine would need to be in excess

of 100% if the hepatic extraction of this drug in man was only 60%, as was found in dogs. Renal metabolism could still account for some of the extrahepatic prilocaine clearance and a study of this would probably be best accomplished using an isolated perfused kidney preparation.

Other possible sites of prilocaine metabolism include the gut, muscle and blood. It has been shown that prilocaine is stable in human whole blood (Appendix 3), implying that no metabolism occurred. To look for gut metabolism of prilocaine, blood prilocaine concentration data is required from the arterial supply to the gut and the venous system draining it. This data was effectively generated in the study of hepatic extraction of prilocaine in dogs, where blood was sampled from the femoral artery and the portal vein. If prilocaine was metabolised by the gut, then the AUC value derived from the arterial blood drug concentration data would be greater than the value derived from the portal venous samples, as less drug would have reached the portal vein. The AUC values obtained from the arterial and portal venous prilocaine concentration data (Table 4-8) gave very similar results (330 $\mu g\ ml^{-1}.min$ - arterial; 313 $\mu g\ ml^{-1}.min$ - portal venous). Although the value for the arterial data is 5% more than the portal venous value, the arterial AUC is based on only nine data points and the similarity between these two AUC values suggests that the gut is not a site of extrahepatic metabolism. There is no data to implicate muscle as a site of prilocaine metabolism. The data presented here indicates that it is

unlikely that there is an individual site of extrahepatic prilocaine metabolism. It is possible then that this extrahepatic clearance involves extremely low levels of prilocaine metabolism in most of the body tissues. Hence, the small pulmonary extraction of prilocaine at steady state blood concentrations in the dog and the 5% difference in arterial and portal venous AUC values may be part of a widespread metabolism of prilocaine throughout the body.

Another explanation for the high clearance value obtained for prilocaine is that this compound could be highly bound in body tissues and released back into the blood very slowly, so as to produce blood drug concentrations less than the minimum detectable limit of the assay. However, this period of drug release would have to last several days to produce an AUC large enough to reduce the clearance value to less than hepatic blood flow. It thus seems unlikely that this occurs. Further work remains to be done to elucidate the mechanisms involved in the extrahepatic clearance of prilocaine.

The results of the pharmacokinetic study in humans help to explain the low central nervous system toxicity of prilocaine. Åström & Persson (1961) demonstrated that prilocaine was less toxic than lignocaine in mice when these drugs were administered by intravenous infusion. Later, work with human volunteers (Eriksson et al, 1966) showed prilocaine to be less toxic than lignocaine after intravenous infusion of these drugs. Toxic reactions were

evaluated by subjective symptoms, eg numbness of the lips and tongue, auditory disturbances and drowsiness, and also symptoms recorded by the experiment monitor, eg slurred speech, ability to answer questions correctly and muscular twitching. Again using intravenous infusions of lignocaine and prilocaine in human volunteers, Eriksson & Persson (1966) demonstrated that greater changes in human electroencephalogram recordings occurred after the lignocaine infusion than after the prilocaine infusion. These differences can be explained by the higher clearance of prilocaine than lignocaine in man (2.42 l min^{-1} and 0.95 l min^{-1} respectively) and its larger volume of distribution (343 l and 212 l respectively). A comparison of the V_c values of prilocaine and lignocaine show a similar difference. The V_c of prilocaine from the human volunteer study (Section 1) was found to be 1.3 l kg^{-1}, compared to the value of 0.8 l kg^{-1} for lignocaine (Boyes et al, 1971). The V_c determined for mepivacaine in this work was also 0.8 l kg^{-1}. These factors will reduce blood concentrations of prilocaine more rapidly than those of lignocaine and thus the possibility of toxic concentrations of prilocaine occurring will be reduced. Although a lower blood concentration of prilocaine could mean a higher brain tissue concentration, it has been shown by Åkerman et al (1966a) that, at the onset of local anaesthetic induced convulsions, brain tissue concentrations of prilocaine were two to three times greater than those of lignocaine.

Another factor contributing to the low toxicity of prilocaine is the ability of the lung to remove this drug from the circulation. Åkerman et al (1966a) found a greater accumulation of prilocaine than lignocaine in lung tissue of rats and this work has been verified by the in vitro studies with rat lungs reported here (Section 2), where prilocaine was found in concentrations two times greater than those of lignocaine in lung tissue. A similar lung uptake of these two compounds in anaesthetised human subjects has also been demonstrated (Section 6). In each subject studied, the blood concentrations of prilocaine were consistently less than those of lignocaine after a single passage through the pulmonary circulation. A comparison with indocyanine green concentrations indicated a reduction of possible peak concentrations of prilocaine and lignocaine by 57% and 41% respectively. This implies that after intravenous injection of these drugs, their lung uptake will cause a significant reduction in the blood concentrations reaching the brain, with those of prilocaine approximately one third less than lignocaine concentrations.

The greater lung uptake and volume of distribution of prilocaine compared to lignocaine is difficult to explain in terms of their physicochemical properties. The lipid solubility of lignocaine is greater than that of prilocaine (Covino & Vassallo, 1976) which suggests it should be more readily taken up by body tissues. This situation also occurs with the other amide type local

anaesthetics. Post et al (1979) found that the accumulation of three of these drugs by rat lung slice incubations to be in the order bupivacaine > etidocaine > lignocaine, although their order of lipid solubility is etidocaine >> bupivacaine >> lignocaine. The effect of the differences in the pKa values for these drugs was nullified in these experiments by incubating the preparations at different pH values to make the ratio of uncharged base to cationic form of the drug equal in all cases. The results of Post et al suggest that differences in uptake may be related to the molecular structure of these drugs. Fowler et al (1976) demonstrated that the chain length of aliphatic amines was a determining factor on the ability of lung tissue to absorb them. Using chain lengths from C_4 to C_{13}, a maximal lung uptake was found with the C_6 amine. Thus, the differences in the molecular structures of the amide type local anaesthetics could be a factor contributing to the differences in distribution of these drugs.

Although lignocaine is bound to plasma protein to a higher degree than prilocaine (64% and 55% respectively), implying that more free prilocaine would be available for uptake by tissues, this difference alone could not account for the difference in lung uptake of these drugs.

As the lung significantly reduces the blood concentration of the amide type local anaesthetics reaching the central nervous system, it is apparent that any factor

which reduces the ability of the lung to perform this function may lower the dose of drug required to produce toxic effects. Lung fibrosis induced in rats has been shown to impair lung accumulation of isoniazid and dexamethasone (Gardiner et al, 1979) and it is probable that other pulmonary diseases will have a similar effect on lung uptake of drugs. The presence of other drugs has been shown to interfere with lung uptake of local anaesthetics, eg imipramine has been shown to displace lignocaine from perfused rat lung preparations and both imipramine and bupivacaine have been shown to inhibit accumulation of lignocaine by rat lung slice preparations (Post et al, 1979).

With the isolated perfused rat lung preparations (Section 2) changing the pH of the perfusion medium appeared to have no effect on the ability of the lung to accumulate lignocaine. Other work on lung uptake of lignocaine, bupivacaine and etidocaine with varying pH has produced contrasting results. Post et al (1979) found a greater lung tissue uptake of these three drugs at pH 8 than at pH 7, using a tissue slice incubation technique. In vivo work with rabbits (Sjöstrand & Widman, 1973) demonstrated much higher lung concentrations of bupivacaine in acidotic animals than in normal animals after the intravenous infusion of this compound. With the in vitro preparations, the increase in the unionised form of the drug at alkaline pH would mean that a greater proportion of the drug in the incubation solution would be available for intracellular

absorption. In the study with normal and acidotic rabbits, the increase in lung concentrations of bupivacaine were attributed to an increase in the overall blood drug concentration combined with an increase in the organ blood flow. It was also suggested that in an acidotic state, changes in the properties of the cellular membrane could occur and the tissue affinity for bupivacaine increased.

A biphasic elimination of both prilocaine and lignocaine was observed with the in situ perfused rat lung preparations (Section 2). A third phase of elimination was seen with the higher doses of these drugs and attributed to drug passing through the pulmonary circulation unaffected by tissue uptake. The two phases of drug elimination from the lung tissue were related to two different sites of uptake for lignocaine and prilocaine. As the terminal phase drug concentrations increased with dose, it was proposed that the drug uptake associated with this phase was dose dependent. The data suggested that as the dose of the local anaesthetic increased, there was some saturation of the sites of drug uptake. At all doses of lignocaine and prilocaine, the terminal phase prilocaine concentrations were significantly greater than those of lignocaine, suggesting a higher initial tissue uptake of prilocaine than lignocaine. These results are comparable to those obtained by Post et al (1978) who demonstrated that the uptake of lignocaine by isolated perfused rat lungs was biphasic in nature. Also, Bertler et al (1978), investigating lung uptake of lignocaine in pigs, found that

at higher doses less of the total dose of drug was absorbed by the lung, indicating saturation of uptake sites. It was proposed by Post et al (1978) that the mechanisms involved in the uptake of lignocaine were intracellular absorption of the unionised form of the drug and binding of the cationic form of the drug to negatively charged sites on the pulmonary endothelial cell surfaces.

The ability of the lung to remove greater amounts of prilocaine than lignocaine from the circulation was demonstrated in both the isolated rat lung preparations and in the anaesthetised human studies. The peak concentrations of prilocaine reaching the brain were found to be one third less than those of lignocaine in human studies (Section 6) and the much higher brain tissue concentrations of prilocaine than lignocaine necessary to produce convulsions in mice (Akerman et al, 1966a) suggest that prilocaine is less likely to cause toxic reactions in clinical use than lignocaine.

In conclusion, it seems that the lungs are functional in absorbing large amounts of the amide type local anaesthetics and play a major role in protecting the central nervous system from toxic concentrations of these drugs. Although it has been shown that lung tissue can metabolise prilocaine <u>in vitro</u>, the intact organ appears to have little or no capability for this metabolism. The high clearance and volume of distribution of prilocaine found in man and animals help to explain the low central nervous system toxicity of this drug.

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A P P E N D I C E S

APPENDIX 1

GENERAL METHOD FOR GLC DETERMINATION OF PRILOCAINE HYDROCHLORIDE AND LIGNOCAINE HYDROCHLORIDE MONOHYDRATE IN RAT LUNG PERFUSATE AND HUMAN BLOOD (A)

GLC DETERMINATION OF PRILOCAINE HYDROCHLORIDE IN HUMAN PLASMA (B), DOG BLOOD (B) AND RABBIT BLOOD (C)

Reagents

Lignocaine HCl BP (Xylotox, Pharmaceutical Manufacturing Co, Bolton, GB)
- 199 $\mu g\ ml^{-1}$ stock solution in 0.1 M HCl

Prilocaine HCl (Citanest, Astra Pharmaceutical Products Inc, Worcester, Mass, USA)
- 335 $\mu g\ ml^{-1}$ stock solution in 0.1 M HCl

Methyl ethyl glycine xylidide HCl (W36004 HCl, Astra Pharmaceutical Products Inc)
- 193.7 $\mu g\ ml^{-1}$ stock solution in 0.1 M HCl

Dichloromethane (AnalaR grade, BDH Chemicals Ltd, Poole, Dorset, GB)

Sodium Hydroxide (AR grade, Koch Light Laboratories, Colbrook, GB)
- 5 M solution in distilled water

Tris (Tris(Hydroxymethyl) methylalanine, AnalaR grade, BDH Chemicals Ltd)
- 2 M solution in distilled water

Absolute alcohol (AR Quality, James Burrough Ltd, London, GB)

Apparatus

10 µl Hamilton Syringe (Microliter No 701, Dyson Instruments, Durham, GB)

11 ml capacity Quickfit round bottomed test tubes with ground glass stoppers and Quickfit 10 ml capacity conical centrifuge tubes (Macfarlane Robson, Glasgow, GB)

Conical centrifuge tubes - 5 ml capacity (Macfarlane Robson, Glasgow, GB)

Reciprocal test tube shaker (Townson & Mercer, Edinburgh, GB)

Vortex mixer (Gallenkamp, Glasgow, GB)

Heated water bath (Gallenkamp, Glasgow, GB)

Chromatography

Instrument - Hewlett Packard Series 402 Gas Chromatograph (Hewlett Packard, South Queensferry, West Lothian, GB)

Detector - Nitrogen selective (rubidium bromide crystal) flame ionisation detector (Hewlett Packard, Model 15161A)

Column and Packing - 6 ft silanised glass U shaped column with an internal diameter of 3/16 in. The column was packed with 3% OV-17 on Chromosorb W-HP 80-100 mesh (Supelco, Canvey Island, Essex, GB) and conditioned at 300°C for 24 hr with nitrogen flowing through the column at approximately 10 ml min^{-1}

Conditions

Oven temperature - 185°C (A), 205°C (B and C)

Flash heater temperature - 250°C

Flame detector temperature - 300°C

Helium (carrier gas) flow rate - 50 ml min^{-1}

Hydrogen flow rate - 30 ml min^{-1}

Air flow rate - approximately 300 ml min^{-1}

Method (A and B)

To 2 ml of sample in an 11 ml test tube were added: 1 ml aqueous W36004 HCl solution (stock solution diluted to a concentration of 3.87 µg ml^{-1} with distilled water), 0.5 ml of 5 M sodium hydroxide solution and 5 ml dichloromethane. The tubes were stoppered and placed horizontally on the reciprocal shaker and shaken for 15 minutes. The tubes were then centrifuged at approximately g x 1200 to separate the aqueous and organic phases. The upper aqueous layer was aspirated off and the remaining dichloromethane decanted into the 10 ml conical centrifuge tubes. These tubes were placed in a water bath at 45°C in a fume cupboard to evaporate off all the dichloromethane. The samples were reconstituted in 20 µl absolute alcohol and 1-4 µl aliquots injected into the gas chromatograph.

Method (C)

To 0.5 ml blood in a centrifuge tube were added: 100 µl of aqueous W36004 HCl solution (stock solution diluted to 19.4 µg ml^{-1} in distilled water), 100 µl 2 M Tris solution and 1 ml dichloromethane. The contents of the tube were mixed on a vortex mixer for 30 sec then the tubes centrifuged at approximately g x 1200 for 15 min. The upper aqueous phase was aspirated off and the remaining dichloromethane decanted into another centrifuge tube. These tubes were placed in a water bath at 45°C in a fume cupboard to evaporate off all the dichloromethane. The samples were reconstituted in 10 µl absolute alcohol and 3-5 µl aliquots injected into the gas chromatograph.

Under the conditions previously stated, the retention times on the column were:

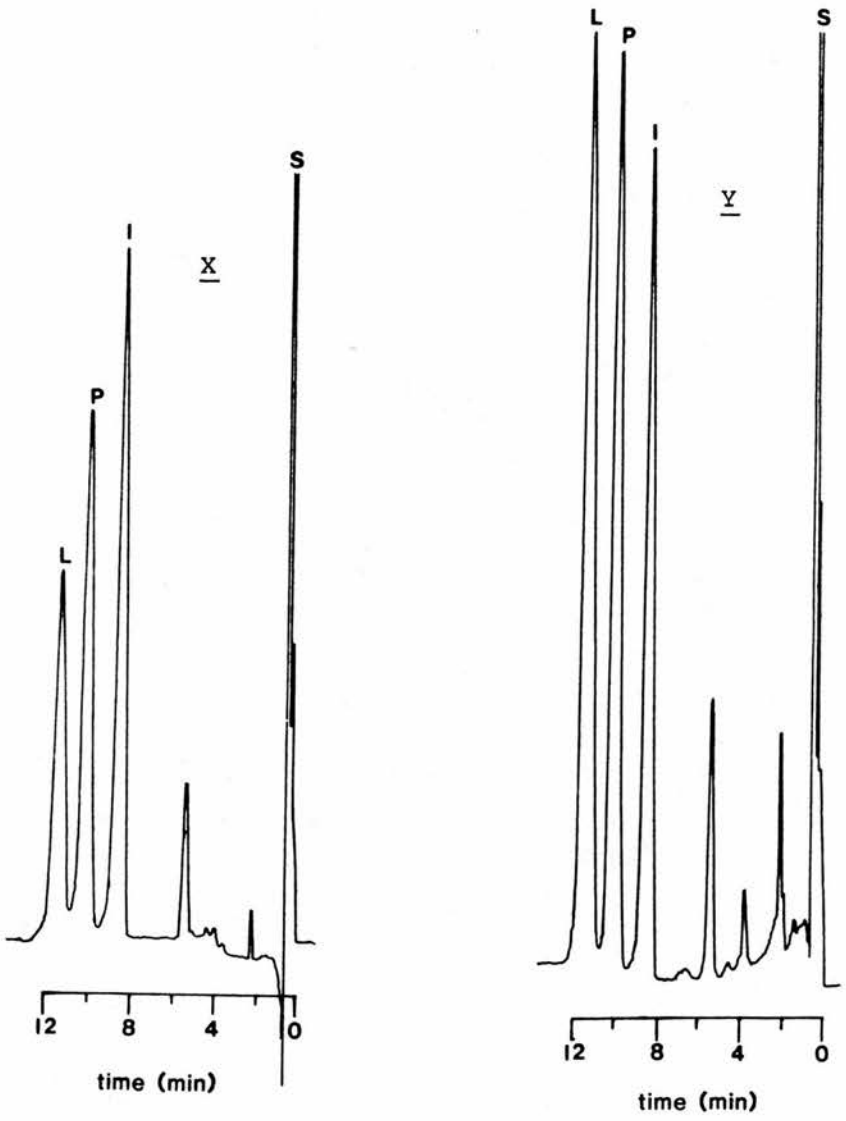
A - W36004 - 8 min
prilocaine - 9.5 min
lignocaine - 11 min

B and C - W36004 - 5 min
prilocaine - 6 min

Chromatograms are shown in Figs A1 and A2.

Standard samples were prepared by 'spiking' 2 ml blank perfusate/blood/plasma samples with microlitre quantities of the prilocaine and/or lignocaine stock solutions and these samples run through the appropriate extraction procedure as described above. Standard samples were run with each set of samples analysed. Sample concentrations of the drugs were calculated from the ratio of the peak height of either prilocaine or lignocaine to the peak height of W36004. Calibration curves were constructed from runs of standard samples of perfusate/blood/plasma.

FIGURE A1



L = LIGNOCAINE P = PRILOCAINE I = W36004 S = SOLVENT

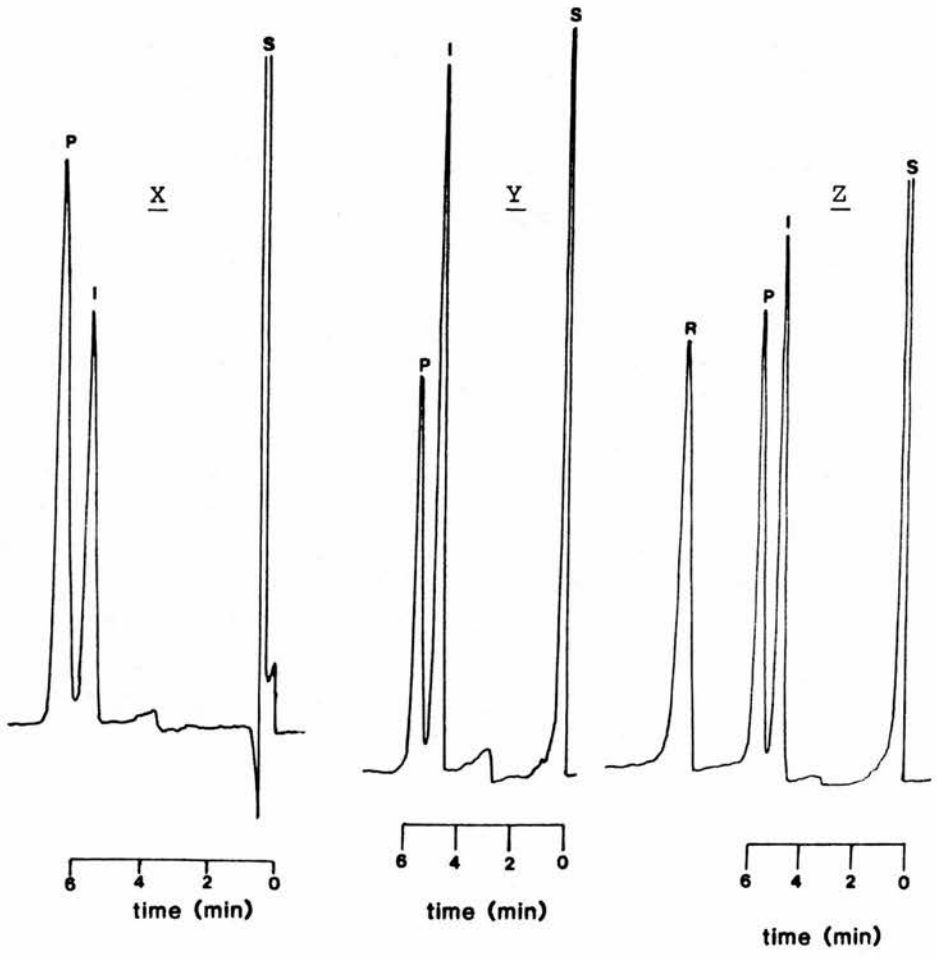
CHROMATOGRAMS OF LIGNOCAINE, PRILOCAINE AND W36004 AFTER EXTRACTION FROM RAT LUNG PERFUSATE (X) AND HUMAN BLOOD (Y)

DRUG CONCENTRATIONS: LIGNOCAINE - 1.46 $\mu g\ ml^{-1}$ (X) ; 3.00 $\mu g\ ml^{-1}$ (Y)
PRILOCAINE - 1.71 $\mu g\ ml^{-1}$ (X) ; 2.47 $\mu g\ ml^{-1}$ (Y)

THERE WERE NO PEAKS IN BLANK PERFUSATE / BLOOD SAMPLES INTERFERING WITH THE DRUG PEAKS

FIGURE A2

CHROMATOGRAMS OF PRILOCAINE AND W36004 AFTER EXTRACTION FROM DOG BLOOD (X), RABBIT BLOOD (Y) AND HUMAN PLASMA (Z)



P = PRILOCAINE I = W36004 S = SOLVENT R = UNIDENTIFIED PEAK FOUND IN MANY HUMAN BLOOD/PLASMA SAMPLES. THIS PEAK DID NOT INTERFERE WITH OTHER DRUG PEAKS.

PRILOCAINE CONCENTRATIONS: 3.16 $\mu g\ ml^{-1}$ (X)
2.33 $\mu g\ ml^{-1}$ (Y)
3.55 $\mu g\ ml^{-1}$ (Z)

The mean, standard deviation and coefficient of variation for series of standard samples were:

A. Rat Lung Perfusate

Standard Sample Concentration: Lignocaine 1.99 μg ml^{-1}
Prilocaine 2.01 μg ml^{-1}

Lignocaine: 2.03, 2.07, 2.01, 2.14, 1.85, 1.98, 1.97, 2.07, 2.13, 1.89 ($\bar{x}$ = 2.01, SD = 0.10, CV = 5%)

Prilocaine: 2.11, 2.22, 2.08, 2.31, 1.99, 1.98, 1.98, 2.07, 2.11, 1.92 ($\bar{x}$ = 2.08, SD = 0.12, CV = 6%)

A. Human Blood

Standard Sample Concentration: Lignocaine 1.99 μg ml^{-1}
Prilocaine 2.01 μg ml^{-1}

Lignocaine: 2.10, 1.76, 1.98, 1.91, 1.84, 1.91, 1.82
($\bar{x}$ = 1.90, SD = 0.11, CV = 6%)

Prilocaine: 2.17, 2.00, 2.07, 1.93, 1.99, 2.13, 2.19
($\bar{x}$ = 2.07, SD = 0.10, CV = 5%)

B. Human Plasma

Standard Sample Concentration: Prilocaine 2.68 μg ml^{-1}

Prilocaine: 2.79, 2.62, 2.58, 2.48, 2.57, 2.60
($\bar{x}$ = 2.61, SD = 0.10, CV = 4%)

B. Dog Blood

Standard Sample Concentration: Prilocaine 1.68 μg ml^{-1}

Prilocaine: 1.65, 1.69, 1.76, 1.70, 1.73, 1.73, 1.74, 1.73, 1.71, 1.76 ($\bar{x}$ = 1.72, SD = 0.03, CV = 2%)

C. Rabbit Blood

Standard Sample Concentration: Prilocaine 2.68 μg ml^{-1}

Prilocaine: 2.50, 2.70, 2.38, 2.60, 2.57
($\bar{x}$ = 2.55, SD = 0.12, CV = 5%)

The drug concentrations were determined as μg ml^{-1} prilocaine hydrochloride or μg ml^{-1} lignocaine hydrochloride monohydrate. Throughout this thesis, these terms have generally been referred to as prilocaine or lignocaine concentrations.

GLC DETERMINATION OF MEPIVACAINE HYDROCHLORIDE IN HUMAN PLASMA

The method was essentially the same as for the determination of prilocaine hydrochloride in human plasma.

The coefficient of variation for a series of standard samples was ± 5% at a concentration of 1 μg ml^{-1}.

APPENDIX 2

DETERMINATION OF THE STABILITY OF PRILOCAINE WHEN FROZEN IN PLASMA

INTRODUCTION

As many of the plasma and blood samples gathered in the course of these experiments were stored frozen, a check on the stability of prilocaine in frozen plasma samples was undertaken.

METHOD

A 100 ml sample of plasma from the Regional Blood Transfusion Service (Royal Infirmary of Edinburgh) was 'spiked' with 1 ml of aqueous prilocaine hydrochloride solution to give a concentration of 3.35 $\mu g\ ml^{-1}$. 3 ml aliquots of the plasma were transferred to 5 ml plain plastic tubes and placed in a deep freeze at approximately $-20^{o}C$. Pairs of samples were analysed for prilocaine content at intervals for seven months, with the analysis of the first pair of samples taking place on the day of preparation of the samples and before they had been frozen. The analytical technique used was the GLC method described in Appendix 1.

RESULTS AND CONCLUSION

The mean prilocaine concentration in each pair of samples is given below:

Time (months)	0	1	2	3	4	7
Prilocaine hydrochloride concentration ($\mu g\ ml^{-1}$)	3.53	3.46	3.54	3.25	3.44	3.39

Although there is slight variation in the results, it would appear that prilocaine is stable when frozen in plasma samples.

APPENDIX 3

DETERMINATION OF THE STABILITY OF PRILOCAINE IN HUMAN BLOOD

INTRODUCTION

Unlike other amide type local anaesthetics, the primary metabolic pathway of prilocaine is by hydrolysis (Geddes, 1965; Åkerman et al, 1966a and b). It is possible that hydrolytic enzymes in the blood could initiate prilocaine metabolism and this experiment was designed to see if this was true.

METHOD

Venous blood samples were withdrawn from two healthy male subjects and transferred to 10 ml plastic lithium heparin tubes (Brunswick, Sherwood Medical Industries, Ballymoney, Co Antrim, N Ireland). For each subject aliquots of 3 ml of blood were transferred to 10 ml glass tubes and 'spiked' with microlitre quantities of aqueous prilocaine hydrochloride solution to give a resultant concentration of 1.12 $\mu g\ ml^{-1}$. Two tubes from each set were immediately frozen and the remaining four tubes were placed in a water bath at $37^{\circ}C$ with a reciprocal shaker. After 30 minutes, two more tubes from each set were removed from the water bath and frozen then the remaining tubes removed and frozen after 60 minutes.

The blood was subsequently assayed for prilocaine content using a GLC technique as described in Appendix 1.

RESULTS AND CONCLUSION

The mean prilocaine concentration data for each pair of samples at the three time periods is given below:

PRILOCAINE HYDROCHLORIDE CONCENTRATIONS IN BLOOD

($\mu g\ ml^{-1}$)

Time (min)	0	30	60
Subject			
1	1.06	1.04	1.06
2	1.21	1.18	1.22

These results indicate that prilocaine is not hydrolised in human blood.

APPENDIX 4

DETERMINATION OF THE WHOLE BLOOD/PLASMA PRILOCAINE CONCENTRATION RATIO

INTRODUCTION

When expressing drug concentration data, it is generally important to indicate if the data refers to whole blood or plasma concentrations. The distribution of a drug between the red blood cells and plasma depends on the extent of plasma protein binding of the drug. In the case of prilocaine, Covino & Vassallo (1976) quoted the percentage plasma protein binding to be 55% and the whole blood/plasma concentration ratio to be 1.0, suggesting that for prilocaine, whole blood and plasma concentration data are interchangeable. This experiment was designed to determine if this was in fact the case.

METHODS

Venous blood samples were withdrawn from two healthy male subjects and transferred to 10 ml plastic lithium heparin tubes (Brunswick, Sherwood Medical Industries, Ballymoney, Co Antrim, N Ireland). Microlitre quantities of aqueous prilocaine hydrochloride solution were added to 4 ml aliquots of the fresh blood in silanised 10 ml glass test tubes to give concentrations of 0.36, 1.78, 3.55 and 7.10 $\mu g\ ml^{-1}$. One sample at each concentration was prepared in both subjects' blood. The blood samples were thoroughly mixed then 1 ml of blood pipetted from the samples and the remaining 3 ml of blood centrifuged to separate the plasma.

1 ml aliquots of the plasma and whole blood were assayed for prilocaine using the GLC technique described in Appendix 1.

RESULTS AND DISCUSSION

The whole blood/plasma concentration ratios are shown below:

Prepared Concentration ($\mu g\ ml^{-1}$)	Subject	Whole Blood Concentration / Plasma Concentration
0.36	1	1.11
0.36	2	0.90
1.78	1	0.95
1.78	2	1.00
3.55	1	1.01
3.55	2	1.02
7.10	1	0.99
7.10	2	1.04
	$\bar{x}$	1.00
	SD	0.06

These results confirm that it is possible to interchange whole blood and plasma prilocaine concentration data.

APPENDIX 5

HEART RATE AND BLOOD PRESSURE
HUMAN VOLUNTEER STUDY

(mean values ± SD)

Time (min)	Heart rate (beats min^{-1})		Systolic BP (mm Hg)		Diastolic BP (mm Hg)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
	PRILOCAINE INFUSION					
-10	77	18	125	13	76	3
-5	74	14	122	14	72	6
0	73	12	127	15	75	9
5	73	14	128	9	81	2
10	72	12	126	12	79	7
12.5	73	12	127	13	81	8
15	72	12	126	13	79	13
20	70	12	125	12	78	11
25	68	13	126	11	77	13
30	71	12	122	13	78	8
40	68	12	125	10	79	4
	MEPIVACAINE INFUSION					
-10	76	5	124	6	71	7
-5	74	7	123	6	71	7
0	76	9	125	3	74	9
5	80	14	126	4	77	11
10	79	15	127	4	82	11
12.5	80	9	127	1	84	11
15	77	9	126	5	81	12
20	75	9	128	6	78	7
25	73	10	127	4	78	6
30	75	7	124	4	77	8
40	75	9	123	6	77	8

APPENDIX 6

PRILOCAINE HYDROCHLORIDE PLASMA CONCENTRATION DATA
HUMAN VOLUNTEER STUDY

SUBJECT	HB	GD	DF	AN	DL	RA	JM	RM	GO	BM	$\bar{x}$	SE
TIME (min)												
5	0.83	0.69	1.49	0.49	0.17	0.98	1.00	0.98	3.31	1.69	1.16	0.28
10	1.43	0.76	2.59	0.98	0.34	3.48	1.69	2.21	4.66	3.12	2.13	0.43
12.5	1.45	1.06	3.26	1.43	1.10	3.70	2.49	1.98	5.27	2.75	2.45	0.43
15	1.03	0.95	1.81	1.50	1.45	2.72	1.77	1.94	3.16	2.16	1.85	0.22
20	0.77	0.63	1.03	0.99	0.95	1.60	1.21	1.29	1.82	1.48	1.18	0.12
25	0.63	0.56	0.68	0.94	0.62	1.09	1.01	1.02	1.39	1.19	0.91	0.09
30	0.55	0.43	0.61	0.91	0.59	0.92	0.93	0.84	1.10	0.98	0.79	0.07
40	0.43	0.35	0.70	0.50	0.45	0.60	0.67	0.64	0.79	0.67	0.58	0.04
60	0.30	0.28	0.44	0.49	0.41	0.34	0.43	0.51	0.57	0.45	0.42	0.03
90	0.26	0.24	0.33	0.12	0.35	0.26	0.39	0.40	0.36	0.25	0.30	0.03
120	0.22	0.19	0.23	0.17	0.27	0.20	0.29	0.24	0.27	0.17	0.23	0.01
180						0.17	0.22	0.16	0.20	0.14	0.18	0.01
240	0.12	0.09	0.11	0.02	0.12	0.07	0.13	0.09	0.10	0.13	0.10	0.01
300						0.08	0.08	0.06	0.04	0.12	0.08	0.01
360						0.04	0.06	0.04	0.02	0.08	0.05	0.01
420						0	0	0	0	0	0	0

APPENDIX 7

MEPIVACAINE HYDROCHLORIDE PLASMA CONCENTRATION DATA
HUMAN VOLUNTEER STUDY

SUBJECT	HB	GD	DF	AN	DL	$\bar{x}$	SE
TIME (min)							
5	1.74	2.28	2.02	1.17	3.55	2.15	0.40
10	2.78	3.04	3.54	1.94	5.40	3.34	0.58
12.5	3.91	2.82	4.05	2.48	7.25	4.10	0.84
15	3.67	1.97	3.48	2.23	4.68	3.21	0.50
20	2.60	1.68	2.64	2.31	4.26	2.70	0.43
25	2.49	1.33	2.60	2.04	3.22	2.34	0.31
30	1.69	1.07	2.24	1.78	3.05	1.97	0.33
40	1.92	0.93	2.13	1.53	2.33	1.77	0.25
60	2.47	0.87	1.87	1.43	2.24	1.78	0.29
90	1.63	0.68	1.56	1.35	1.65	1.37	0.18
120	1.54	0.68	1.30	1.01	1.46	1.20	0.16
240	0.86	0.18	0.68	0.64	0.69	0.61	0.11

APPENDIX 8

INDIVIDUAL PHARMACOKINETIC DATA FOR PRILOCAINE AFTER INTRAVENOUS INFUSION
HUMAN VOLUNTEER STUDY

SUBJECT	HB	GD	DF	AN	DL	RA	JM	RM	GO	BM	$\bar{x}$	±SD
VARIABLE												
α (min^{-1})	0.080	0.105	0.167	0.061	0.105	0.093	0.087	0.089	0.148	0.070	0.101	0.033
β (min^{-1})	0.0052	0.0057	0.0081	0.0146	0.0068	0.0069	0.0072	0.0083	0.0109	0.0049	0.0079	0.0029
$t_{\frac{1}{2}\alpha}$ (min)	8.7	6.6	4.1	11.3	6.6	7.5	8.0	7.8	4.7	10.0	7.5	2.2
$t_{\frac{1}{2}\beta}$ (min)	134	122	86	43	102	101	96	83	64	141	97	30
k_{10} (min^{-1})	0.020	0.021	0.047	0.026	0.026	0.044	0.025	0.028	0.081	0.028	0.035	0.019
k_{21} (min^{-1})	0.021	0.029	0.029	0.034	0.028	0.015	0.025	0.027	0.020	0.012	0.024	0.006
k_{12} (min^{-1})	0.044	0.061	0.178	0.015	0.058	0.041	0.044	0.043	0.101	0.034	0.062	0.046
A ($\mu g\ ml^{-1}$)	1.48	1.24	4.08	1.13	2.17	4.63	2.38	2.32	7.62	3.13	3.02	1.99
B ($\mu g\ ml^{-1}$)	0.39	0.37	0.61	0.84	0.58	0.46	0.68	0.68	1.05	0.40	0.61	0.22
AUC_C ($\mu g\ ml^{-1}.min$)	94	77	107	76	106	116	122	108	148	127	108	22
AUC_M ($\mu g\ ml^{-1}.min$)	97	74	114	71	94	117	122	113	155	134	109	26

continued overleaf

APPENDIX 8 (continued)

SUBJECT		HB	GD	DF	AN	DL	RA	JM	RM	GO	BM	$\bar{x}$	±SD
VARIABLE													
Cl_C	(l min^{-1})	2.66	3.25	2.34	3.29	2.36	2.16	2.05	2.31	1.69	1.97	2.41	0.52
	(ml min^{-1} kg^{-1})	41.6	42.2	39.0	51.4	28.7	30.0	30.6	37.9	25.2	27.7	35.4	8.3
Cl_M	(l min^{-1})	2.58	3.38	2.19	3.52	2.66	2.14	2.05	2.21	1.61	1.87	2.42	0.62
	(ml min^{-1} kg^{-1})	40.3	43.9	36.5	55.0	32.4	29.7	30.6	36.2	24.0	26.3	35.5	9.2
V_B	(l)	501	597	312	241	392	310	285	267	148	381	343	130
	(l kg^{-1})	6.6	7.8	5.2	3.8	4.8	4.3	4.3	4.4	2.2	5.4	4.9	1.5
V_c	(l)	134	155	53	127	91	49	82	83	29	71	87	40
	(l kg^{-1})	1.8	2.0	0.9	2.0	1.1	0.7	1.2	1.4	0.4	1.0	1.3	0.5
V_p	(l)	367	442	259	114	301	261	203	184	119	310	256	105
	(l kg^{-1})	4.8	5.7	4.3	1.8	3.7	3.6	3.0	3.0	1.8	4.4	3.6	1.3

APPENDIX 9

INDIVIDUAL PHARMACOKINETIC DATA FOR MEPIVACAINE AFTER INTRAVENOUS INFUSION
HUMAN VOLUNTEER STUDY

SUBJECT	HB	GD	DF	AN	DL	$\bar{x}$	±SD
VARIABLE							
α (min^{-1})	0.136	0.169	0.128	0.074	0.100	0.121	0.036
β (min^{-1})	0.0043	0.0084	0.0058	0.0045	0.0064	0.0059	0.0016
k_{10} (min^{-1})	0.011	0.025	0.012	0.007	0.018	0.015	0.007
k_{21} (min^{-1})	0.056	0.057	0.065	0.045	0.035	0.052	0.012
k_{12} (min^{-1})	0.074	0.096	0.058	0.026	0.053	0.061	0.026
$t_{½}\alpha$ (min)	5.1	4.1	5.4	9.4	6.9	6.2	2.1
$t_{½}\beta$ (min)	162	83	120	154	108	125	32
A ($\mu g\ ml^{-1}$)	3.60	3.04	2.80	1.28	6.66	3.48	1.98
B ($\mu g\ ml^{-1}$)	2.28	1.31	2.54	1.80	2.95	2.18	0.64
AUC_C ($\mu g\ ml^{-1}.min$)	559	175	463	417	526	428	152
AUC_M ($\mu g\ ml^{-1}.min$)	579	180	469	444	530	440	155
Cl_C ($l\ min^{-1}$)	0.45	1.43	0.54	0.60	0.48	0.70	0.41
Cl_C ($ml\ min^{-1}\ kg^{-1}$)	5.9	18.6	9.0	9.4	5.8	9.7	5.2
Cl_M ($l\ min^{-1}$)	0.43	1.39	0.53	0.56	0.47	0.68	0.40
Cl_M ($ml\ min^{-1}\ kg^{-1}$)	5.7	18.3	8.9	8.8	5.8	9.5	5.2
V_B (l)	104	170	93	133	74	115	38
V_B ($l\ kg^{-1}$)	1.4	2.2	1.6	2.1	0.9	1.6	0.5
V_c (l)	43	57	47	81	26	51	20
V_c ($l\ kg^{-1}$)	0.6	0.8	0.8	1.3	0.3	0.8	0.4
V_p (l)	61	113	46	52	48	64	28
V_p ($l\ kg^{-1}$)	0.8	1.5	0.8	0.8	0.6	0.9	0.3

APPENDIX 10

KREB'S RINGER BICARBONATE BUFFER

NaCl	-	7.9 g
KCl	-	0.35 g
$CaCl_2.2H_2O$	-	0.37 g
$MgSO_4.7H_2O$	-	0.286 g
$NaHCO_3$	-	2.09 g
KH_2PO_4	-	0.16 g
Glucose	-	1 g

Dissolved in 1 litre of distilled water

APPENDIX 11

TYRODES BUFFER

NaCl	-	8.0 g
KCl	-	0.2 g
$MgCl_2$	-	0.1 g
$CaCl_2.2H_2O$	-	0.2 g
NaH_2PO_4	-	0.05 g
$NaHCO_3$	-	1.0 g
Glucose	-	1.0 g

Dissolved in 1 litre of distilled water

APPENDIX 12

HEART RATE AND BLOOD PRESSURE IN DOGS
PULMONARY EXTRACTION OF PRILOCAINE

(mean values ± SD)

Time (min)	Heart rate (beats min^{-1})		Systolic BP (mm Hg)		Diastolic BP (mm Hg)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
0	159	22	104	11	76	16
20	163	23	105	9	79	15
40	163	21	109	12	82	17
60	164	22	114	21	85	21
80	157	21	105	32	76	29
90	153	23	106	29	75	31
100	150	22	110	24	81	23
105	151	20	113	24	83	23
110	150	19	119	16	86	16
115	150	19	116	19	83	19
120	150	18	116	19	86	21
130	147	16	119	22	90	23
145	144	11	119	21	91	21
160	143	11	118	23	91	21
190	141	11	120	23	93	20
220	142	13	123	26	98	25

APPENDIX 13

BLOOD GAS ANALYSIS OF DOG ARTERIAL BLOOD SAMPLES

DOG	1					2					3				
Sample	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
pH		7.22	7.19	7.13	7.23	7.18	7.25	7.24	7.14	7.27	7.03	7.05	7.07	7.09	7.16
pCO_2 (kPa)		7.2	8.2	9.6	7.1	8.3	6.8	7.2	9.2	6.6	9.8	9.6	9.3	9.3	8.3
pO_2 (kPa)		18.9	19.3	20.1	22.9	29.0	16.3	14.7	12.7	18.2	18.9	16.9	17.3	16.4	20.8
(HCO_3^-) (mM l^{-1})		20	20	20	20	23	22	23	23	22	19	20	20	21	22

DOG	4					5					6				
Sample	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
pH	7.26	7.32	7.33	7.33	7.35	7.23	7.27	7.21	7.27	7.20	7.14	7.14	7.13	7.11	7.13
pCO_2 (kPa)	7.0	6.2	6.3	6.0	6.0	7.7	6.4	7.2	5.9	7.6	9.7	9.9	10.1	10.7	10.5
pO_2 (kPa)	20.3	15.2	15.5	16.6	17.9	15.0	17.4	16.9	15.4	19.1	20.6	16.6	15.4	15.4	18.0
(HCO_3^-) (mM l^{-1})	23	23	24	24	25	24	22	21	20	22	24	24	24	24	25

Normal values (Feigl & d'Alecy, 1972): pH = 7.34 to 7.51 pCO_2 = 4.1 to 5.7 kPa
pO_2 = 10.3 to 13.6 kPa (HCO_3^-) = 23.5 mM l^{-1}

APPENDIX 14

PRILOCAINE HYDROCHLORIDE BLOOD CONCENTRATION DATA FOR DOGS 1, 2 AND 3

($\mu g\ ml^{-1}$)

TIME (min)	DOG 1		DOG 2		DOG 3	
	ART	MV	ART	MV	ART	MV
20	3.60	3.09	4.29	2.14	5.49	8.30
40	2.26	2.21	2.37	1.32	3.55	5.70
60	2.35	2.35	2.45	1.48	3.69	5.59
62	2.40	2.33	2.50	1.64	3.66	5.93
64	2.41	2.23	2.45	1.46	3.76	5.58
66	2.40	2.19	2.48	X	3.75	5.31
68	2.39	2.30	2.42	1.44	3.43	5.67
70	2.36	2.32	2.50	1.39	3.68	5.67
72	2.34	2.49	2.51	1.49	3.67	6.00
76	2.31	2.37	2.42	1.37	3.79	5.83
78	2.44	2.26	2.58	1.51	3.72	6.05
80	2.46	2.31	2.67	1.69	3.72	5.90
82	2.39	2.27	2.71	1.70	3.76	6.04
84	2.49	2.31	2.74	1.82	3.76	5.96
86	2.52	2.52	2.79	1.94	3.80	6.04
88	2.64	2.47	2.79	1.85	3.93	5.92
90	2.65	2.54	2.97	2.14	3.86	5.84
95	2.69	2.56	3.02	1.86	3.91	5.83
100	2.66	2.28	2.97	2.07	3.97	4.62
102	1.91	1.76	1.93	1.74	2.92	3.20
104	X	1.65	1.73	1.41	2.60	2.96
106	1.57	1.49	1.62	1.29	2.37	2.65
108	1.47	1.46	1.41	1.20	2.15	2.44
110	1.36	1.34	1.36	1.05	1.96	2.33
115	1.15	1.14	1.20	0.88	1.64	1.96
120	1.13	1.13	1.02	0.82	1.42	1.73
130	0.89	0.91	0.83	0.68	1.12	1.41
145	0.70	0.63	0.66	0.51	0.86	1.15
160	0.57	0.53	0.53	0.41	0.66	0.91
190	0.37	0.37	0.37	0.31	0.46	0.62
220	0.29	0.25	0.28	0.20	0.34	0.49

ART = Arterial concentrations
MV = Mixed venous concentrations
X = No sample obtained

APPENDIX 15

INDIVIDUAL PHARMACOKINETIC DATA FOR PRILOCAINE AFTER INTRAVENOUS INFUSION IN DOGS

MIXED VENOUS SAMPLES

DOG	1	2	3	4	5	6	DOGS 4-6 $\bar{x}$	DOGS 4-6 SD
VARIABLE								
A ($\mu g\ ml^{-1}$)	4.91	8.55	18.82	14.48	15.16	12.10	13.91	1.61
B ($\mu g\ ml^{-1}$)	1.99	1.55	3.14	2.16	1.09	1.91	1.72	0.56
AUC_C ($\mu g\ ml^{-1}.min$)	268	279	509	432	345	365	381	46
AUC_M ($\mu g\ ml^{-1}.min$)	333	241	772	539	370	467	459	85
Cl_C ($ml\ min^{-1}\ kg^{-1}$)	42.6	49.7	43.0	50.7	38.9	42.2	43.9	6.1
Cl_M ($ml\ min^{-1}\ kg^{-1}$)	34.3	57.5	28.4	40.6	36.3	33.0	36.6	3.8
Cl_{ss} ($ml\ min^{-1}\ kg^{-1}$)	37.3	63.5	28.9	44.6	45.4	38.1	42.7	4.0
V_B ($l\ kg^{-1}$)	3.2	5.9	2.6	4.7	5.5	3.7	4.6	0.9
V_c ($l\ kg^{-1}$)	1.7	1.4	1.0	1.3	0.8	1.1	1.1	0.3
V_p ($l\ kg^{-1}$)	1.5	4.3	1.6	3.4	4.7	2.6	3.6	1.1
$t_{½}\beta$ (min)	60	71	64	80	105	78	88	15
$t_{½}\alpha$ (min)	13.5	9.7	8.0	8.8	8.2	8.6	8.5	0.3
β (min^{-1})	0.0116	0.0097	0.0108	0.0087	0.0066	0.0089	0.0081	0.0013
α (min^{-1})	0.051	0.072	0.086	0.079	0.084	0.080	0.081	0.003
k_{10} (min^{-1})	0.026	0.036	0.043	0.039	0.047	0.038	0.040	0.006
k_{21} (min^{-1})	0.023	0.019	0.022	0.018	0.012	0.019	0.016	0.004
k_{12} (min^{-1})	0.014	0.026	0.032	0.031	0.032	0.032	0.032	0.001
Weight (kg)	18.2	15.0	9.5	9.5	15.5	13.5		

continued overleaf

APPENDIX 15 (continued)

ARTERIAL SAMPLES

DOG	1	2	3	4	5	6	TOTAL DATA $\bar{x}$	TOTAL DATA SD	DOGS 4-6 $\bar{x}$	DOGS 4-6 SD
VARIABLE										
A ($\mu g\ ml^{-1}$)	7.79	9.99	18.18	18.49	18.58	12.75	14.30	4.78	16.61	3.34
B ($\mu g\ ml^{-1}$)	2.01	1.38	2.21	2.36	1.23	2.00	1.87	0.46	1.86	0.58
AUC_C ($\mu g\ ml^{-1}.min$)	290	313	453	495	352	370	379	80	406	78
AUC_M ($\mu g\ ml^{-1}.min$)	352	382	521	538	364	468	438	82	457	21
Cl_C ($ml\ min^{-1}\ kg^{-1}$)	39.4	44.3	48.3	44.2	38.1	43.8	43.0	3.7	42.0	3.4
Cl_M ($ml\ min^{-1}\ kg^{-1}$)	32.5	36.3	42.0	40.7	36.9	32.9	36.9	3.9	36.8	3.9
Cl_{ss} ($ml\ min^{-1}\ kg^{-1}$)	35.7	40.1	44.8	45.3	45.3	40.2	41.9	3.9	43.6	2.9
V_B ($l\ kg^{-1}$)	3.0	4.3	4.0	4.6	4.9	3.6	4.1	0.7	4.4	0.7
V_c ($l\ kg^{-1}$)	1.2	1.2	1.1	1.1	0.7	1.0	1.1	0.2	0.9	0.2
V_p ($l\ kg^{-1}$)	1.8	3.1	2.9	3.5	4.2	2.6	3.0	0.8	3.4	0.8
$t_{½\beta}$ (min)	62	83	67	78	91	75	76	11	81	9
$t_{½\alpha}$ (min)	9.7	10.3	9.1	8.2	7.1	8.3	8.8	1.2	7.9	0.7
β (min^{-1})	0.0111	0.0084	0.0104	0.0089	0.0076	0.0092	0.0093	0.0013	0.0086	0.0009
α (min^{-1})	0.071	0.067	0.076	0.084	0.098	0.084	0.080	0.011	0.089	0.008
k_{10} (min^{-1})	0.034	0.036	0.045	0.043	0.056	0.040	0.042	0.008	0.046	0.009
k_{21} (min^{-1})	0.024	0.016	0.018	0.017	0.013	0.019	0.018	0.004	0.017	0.003
k_{12} (min^{-1})	0.025	0.024	0.024	0.033	0.036	0.034	0.029	0.006	0.034	0.002

APPENDIX 16

HEART RATE AND BLOOD PRESSURE IN DOGS
HEPATIC EXTRACTION OF PRILOCAINE

(mean values ± SD)

Time (min)	Heart rate (beats min^{-1})		Systolic BP (mm Hg)		Diastolic BP (mm Hg)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
0	159	19	181	36	132	16
5	159	19	177	34	124	13
10	159	19	167	34	118	13
15	155	21	164	31	115	13
19	158	21	165	26	119	13
25	158	21	162	28	119	17
30	158	21	167	34	120	18
40	157	22	167	25	119	15
55	153	21	162	26	117	13
70	157	28	159	21	113	13
90	153	24	154	20	107	13
120	148	21	150	16	107	14
150	146	22	153	27	107	14
180	148	24	154	24	107	14
210	144	24	150	32	105	24
240	147	30	146	35	105	23

APPENDIX 17

PRILOCAINE HYDROCHLORIDE BLOOD CONCENTRATION DATA FOR DOGS 8, 9 AND 10

($\mu g\ ml^{-1}$)

TIME (min)	DOG 8			DOG 9			DOG 10		
	PV	HV	A	PV	HV	A	PV	HV	A
5	4.68	3.97	13.13	4.60	4.81	9.47	4.28	0.50	7.09
10	8.94	5.74	15.49	8.29	6.49	12.33	6.37	1.44	9.99
15	11.77	5.47	15.42	9.85	7.45	13.23	8.13	1.42	12.03
17	10.13			9.25			6.31		
19	8.91			7.35			5.51		
21	7.40	2.22	4.17	6.30	3.06	4.12	4.03	0.91	3.16
23	6.24			5.30			3.53		
25	5.35			4.91			3.16		
30	3.91	1.15	2.49	3.42	1.72	1.91	2.33	0.51	1.66
40	2.33	0.85	1.62	1.98	1.09	1.33	1.61	0.29	1.12
55	1.48			1.14			1.09		
70	1.04	0.47	1.03	0.81	0.48	0.81	0.83	0.10	0.63
90	0.80			0.46			0.57		
120	0.58	0.23	0.59	0.32	0.24	0.35	0.37	0.08	0.34
150	0.37			0.24			0.24		
180	0.35	0.14	0.39	0.18	0.13	0.20	0.24	0	0.13
210	0.23			0.12			0.16		
240	0.20			0.10			0.12		

PV = Portal venous samples
HV = Hepatic venous samples
A = Arterial samples

APPENDIX 18

INDIVIDUAL PHARMACOKINETIC DATA FOR PRILOCAINE
AFTER INTRAVENOUS INFUSION IN DOGS

DOG	8	9	10	$\bar{x}$	SD
VARIABLE					
A ($\mu g\ ml^{-1}$)	16.09	12.09	7.53	11.90	4.28
B ($\mu g\ ml^{-1}$)	1.62	1.05	1.16	1.28	0.30
AUC_C ($\mu g\ ml^{-1}.min$)	378	286	237	300	72
AUC_M ($\mu g\ ml^{-1}.min$)	383	301	255	313	65
Cl_C ($ml\ min^{-1}\ kg^{-1}$)	27.4	35.2	42.4	35.0	7.5
Cl_M ($ml\ min^{-1}\ kg^{-1}$)	27.0	33.5	39.4	33.3	6.2
V_B ($l\ kg^{-1}$)	2.9	3.4	4.3	3.5	0.7
V_c ($l\ kg^{-1}$)	0.6	0.8	1.2	0.9	0.3
V_p ($l\ kg^{-1}$)	2.3	2.6	3.1	2.7	0.4
k_{10} (min^{-1})	0.047	0.046	0.037	0.043	0.006
k_{21} (min^{-1})	0.016	0.015	0.017	0.016	0.001
k_{12} (min^{-1})	0.026	0.015	0.020	0.020	0.005
$t_{½}\beta$ (min)	75	67	71	71	3.8
$t_{½}\alpha$ (min)	8.8	10.5	10.9	10.1	1.1
α (min^{-1})	0.076	0.066	0.063	0.069	0.008
β (min^{-1})	0.0093	0.0104	0.0098	0.0098	0.0006

APPENDIX 19

PRILOCAINE HYDROCHLORIDE BLOOD CONCENTRATION DATA
FOR NORMAL ANAESTHETISED RABBITS

($\mu g\ ml^{-1}$)

	Arterial					Venous				
RABBIT	1	2	3	$\bar{x}$	SD	1	2	3	$\bar{x}$	SD
TIME (min)										
5	6.33	2.78	3.66	4.26	1.85	1.32	0.79	1.97	1.36	0.59
10	7.32	4.79	4.48	5.53	1.56	2.52	1.97	1.99	2.16	0.31
15	7.27	6.43	5.07	6.26	1.11	3.96	3.30	2.82	3.36	0.57
17	3.45	2.43	2.34	2.74	0.62	3.61	3.03	2.84	3.16	0.40
19	2.87	1.93	1.83	2.21	0.57	3.59	2.17		2.88	
21	2.28	1.92	1.43	1.88	0.43	3.32		2.29	2.81	
25	1.93	1.72	1.44	1.70	0.25	3.02	2.07	1.95	2.35	0.59
30	1.47	1.49	1.56	1.51	0.05	2.88	1.80	1.73	2.14	0.64
40	1.77	1.40	1.66	1.61	0.19	2.23	1.56	1.46	1.75	0.42
55	1.53	1.16	0.99	1.23	0.28	1.80	1.32	1.14	1.42	0.34
70	1.26	1.10	0.84	1.07	0.21	1.46	1.15	0.93	1.18	0.27
100	1.00	0.94	0.67	0.87	0.18	1.12	0.93	0.80	0.95	0.16
130	0.80	0.79	0.58	0.72	0.12	0.87	0.75	0.65	0.76	0.11
160	0.71	0.71	0.50	0.64	0.12	0.73	0.71	0.58	0.67	0.08
190	0.53	0.68	0.46	0.56	0.11	0.57	0.71	0.48	0.59	0.12
220	0.57		0.32	0.45		0.62		0.47	0.55	
250	0.47		0.23	0.35		0.55			0.55	

APPENDIX 20

INDIVIDUAL PHARMACOKINETIC DATA FOR PRILOCAINE AFTER INTRAVENOUS INFUSION IN RABBITS

VENOUS SAMPLES

RABBIT	1	2	3	$\bar{x}$	SD
VARIABLE					
A ($\mu g\ ml^{-1}$)	2.58	2.22	2.43	2.41	0.18
B ($\mu g\ ml^{-1}$)	2.31	1.38	1.22	1.64	0.59
AUC_C ($\mu g\ ml^{-1}.min$)	348	375	296	340	40
AUC_M ($\mu g\ ml^{-1}.min$)	345	385	305	345	40
Cl_C ($ml\ min^{-1}\ kg^{-1}$)	27.2	26.7	32.1	28.7	3.0
Cl_M ($ml\ min^{-1}\ kg^{-1}$)	27.4	26.0	31.1	28.2	2.6
V_B ($l\ kg^{-1}$)	3.5	6.5	6.7	5.6	1.8
V_c ($l\ kg^{-1}$)	1.9	2.8	2.6	2.4	0.5
V_p ($l\ kg^{-1}$)	1.6	3.7	4.1	3.1	1.3
k_{10} (min^{-1})	0.014	0.010	0.012	0.012	0.002
k_{21} (min^{-1})	0.030	0.024	0.022	0.025	0.004
k_{12} (min^{-1})	0.018	0.027	0.027	0.024	0.005
$t_{½}\alpha$ (min)	12.8	12.3	12.1	12.4	0.4
$t_{½}\beta$ (min)	90	169	144	134	40
α (min^{-1})	0.054	0.056	0.057	0.056	0.002
β (min^{-1})	0.0077	0.0041	0.0048	0.0055	0.0019

continued overleaf

APPENDIX 20 (continued)

ARTERIAL SAMPLES

RABBIT	1	2	3	$\bar{x}$	SD
VARIABLE					
A ($\mu g\ ml^{-1}$)	27.88	3.02	2.06	10.99	14.64
B ($\mu g\ ml^{-1}$)	1.63	1.38	1.31	1.44	0.17
AUC_C ($\mu g\ ml^{-1}.min$)	387	363	223	324	89
AUC_M ($\mu g\ ml^{-1}.min$)	410	401	288	366	68
Cl_C ($ml\ min^{-1}\ kg^{-1}$)	24.4	27.5	42.6	31.5	9.7
Cl_M ($ml\ min^{-1}\ kg^{-1}$)	23.1	24.9	33.0	27.0	5.3
V_B ($l\ kg^{-1}$)	4.5	6.7	6.4	5.9	1.2
V_c ($l\ kg^{-1}$)	0.3	2.3	2.8	1.8	1.3
V_p ($l\ kg^{-1}$)	4.2	4.4	3.6	4.1	0.4
k_{10} (min^{-1})	0.076	0.012	0.015	0.034	0.036
k_{21} (min^{-1})	0.024	0.034	0.035	0.031	0.006
k_{12} (min^{-1})	0.243	0.058	0.036	0.112	0.114
$t_{\frac{1}{2}}\alpha$ (min)	2.0	6.9	8.7	5.9	3.5
$t_{\frac{1}{2}}\beta$ (min)	130	167	104	134	32
α (min^{-1})	0.338	0.101	0.080	0.173	0.143
β (min^{-1})	0.0054	0.0041	0.0067	0.0054	0.0013

APPENDIX 21

PRILOCAINE HYDROCHLORIDE BLOOD CONCENTRATION DATA FOR ANEPHRIC ANAESTHETISED RABBITS

($\mu g\ ml^{-1}$)

RABBIT	1	2	3	4
TIME (min)				
5	1.30		1.52	2.61
10	2.28	6.69	2.72	4.14
15	3.60	10.25		4.17
17	3.44	9.88	3.55	3.58
19	3.41	9.42	2.78	2.40
21	3.54	8.83	2.48	2.37
25	3.24	8.22	1.98	1.66
30	3.14	8.06	1.50	1.50
40	3.11	7.83	1.05	1.08
55	3.09	6.90	0.76	0.82
70	2.98	6.61	0.60	0.67
100	2.44	5.01	0.38	0.39
130	1.73	4.63	0.27	0.36
160	1.33		0.23	0.33
190	1.06		0.14	0.21
220	0.93		0.10	0.22
250	0.87		0.09	0.17

APPENDIX 22

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PHARMACOKINETIC AND CLINICAL PHARMACOLOGICAL STUDIES WITH MEPIVACAINE AND PRILOCAINE

G. R. ARTHUR, D. H. T. SCOTT, R. N. BOYES AND D. B. SCOTT

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G. R. ARTHUR, D. H. T. SCOTT, R. N. BOYES AND D. B. SCOTT

SUMMARY

The tolerance and pharmacokinetic properties of mepivacaine and prilocaine were compared following i.v. infusion of 250 mg (0.88 and 0.97 mmol respectively) of each drug in five healthy volunteers. Side-effects were minor and occurred in only two subjects during the infusion of mepivacaine. Plasma concentrations of mepivacaine were greater in each subject than the corresponding values for prilocaine. The elimination half-life of mepivacaine was generally longer than that for prilocaine, whereas the total body clearance of prilocaine was consistently greater than the corresponding value for mepivacaine. For each subject the clearance of prilocaine substantially exceeded normal heptic blood flow and therefore an extra-hepatic site of metabolism of prilocaine has been postulated.

Many studies have been carried out, in both patients and volunteers, in which the detailed pharmacokinetics of certain local anaesthetic agents have been measured and attempts have been made to associate blood or plasma concentrations with pharmacological effects of these drugs (Boyes et al., 1971; Reynolds, 1971b; Scott, 1975). Although early clinical studies suggested smaller blood concentrations and a more rapid clearance for prilocaine than for lignocaine, a more detailed evaluation of the pharmacokinetics of prilocaine has not been carried out in man. Although mepivacaine has been the subject of pharmacokinetic evaluation (Reynolds, 1971a; Tucker and Mather, 1975) there has been no systematic evaluation of the relationship between blood concentration of the drug and its toxic side-effects. This current study compared the pharmacokinetics and clinical effects of both prilocaine and mepivacaine following i.v. administration in a group of volunteers.

METHODS

The study was approved by the appropriate hospital ethics committee. After being informed in detail of the experiments, five healthy males (age 24–35 yr) consented to participate. On two occasions, separated by at least 7 days, each volunteer received, over 12.5 min, i.v. infusion of 250 mg of either mepivacaine HCl (0.88 mmol) or prilocaine HCl (0.97 mmol) in 50 ml saline. The experiment was carried out in a double-blind manner and the infusions were administered in a random sequence. Venous blood samples were taken during the infusion and at frequent intervals for 4 h thereafter. Systemic arterial pressure was monitored during the course of the experiment and the e.c.g. was tape-recorded continuously for 50 min starting 10 min before commencing the infusions.

The venous blood samples were collected into heparinized tubes, centrifuged, and the separated plasma stored in a freezer until analysis. Plasma concentrations (expressed as free base) of mepivacaine and prilocaine were determined using a gas chromatographic procedure similar to that described by Tucker (1970). Under the conditions employed the minimum detectable limit for both drugs was 0.05 μg ml^{-1} and there was a coefficient of variation of $\pm 5\%$ at a concentration of 1 μg ml^{-1}.

The plasma concentrations of prilocaine and mepivacaine for each volunteer were subjected to manual "curve stripping" and the resulting mathematical constants were used for pharmacokinetic analysis (see Appendix). From these data the elimination half-life ($T_{\frac{1}{2}}$), area under the plasma drug concentration curve (AUC_c) and total body clearance ($\dot{V}$) of drug could be derived. The area under the plasma concentration curve was also measured geometrically (AUC_m) as a means of checking the pharmacokinetic calculations.

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TABLE I. *Pharmacokinetic data for mepivacaine after i.v. infusion in five volunteers. α is the slope of the rapid distribution phase of the drug; β is the slope of the slower elimination phase of the drug; A and B the intercept values of the α and β phases on the plasma drug concentration axis at time (t) = 0 (values corrected for infusion time—see Appendix, equations (2) and (3)); $T_{\frac{1}{2}}$ the elimination half-life of the drug; AUC_c and AUC_m the calculated and measured areas under the plasma drug concentration curve (see Appendix); $\dot{V}$ the total body clearance of the drug from the body*

	Volunteer no.						
	1	2	3	4	5	Mean	SD
α (min^{-1})	0.136	0.169	0.128	0.074	0.100	0.101	(±0.066)
β (min^{-1})	0.0043	0.0084	0.0058	0.0045	0.0064	0.0059	(±0.0016)
A (μg ml^{-1})	3.6	3.04	2.80	1.28	6.66	3.48	(±1.98)
B (μg ml^{-1})	2.28	1.31	2.54	1.80	2.95	2.18	(±0.64)
$T_{\frac{1}{2}}$ (min)	162	83	120	154	108	125	(±32)
AUC_c (μg ml^{-1} min^{-1})	559	175	463	417	526	428	(±152)
AUC_m (μg ml^{-1} min^{-1})	579	180	469	444	530	440	(±155)
$\dot{V}$ (litre min^{-1})	0.45	1.43	0.54	0.60	0.48	0.70	(±0.41)

TABLE II. *Pharmacokinetic data of prilocaine after i.v. infusion in five volunteers*

	Volunteer no.						
	1	2	3	4	5	Mean	SD
α (min^{-1})	0.080	0.105	0.364	0.061	0.105	0.143	(±0.125)
β (min^{-1})	0.0052	0.0057	0.0107	0.146	0.0068	0.0086	(±0.0040)
A (μg ml^{-1})	1.48	1.24	8.29	1.13	2.17	2.86	(±3.06)
B (μg ml^{-1})	0.39	0.37	0.77	0.84	0.58	0.59	(±0.21)
$T_{\frac{1}{2}}$ (min)	134	122	65	43	102	93	(±38)
AUC_c (μg ml^{-1} min^{-1})	94	77	95	76	106	90	(±13)
AUC_m (μg ml^{-1} min^{-1})	97	74	114	71	94	90	(±18)
$\dot{V}$ (litre min^{-1})	2.66	3.25	2.64	3.29	2.36	2.84	(±0.41)

RESULTS

No significant alterations in arterial pressure or e.c.g. were observed during any of the infusions . The only subjective signs of toxicity were noted by two of the volunteers who reported numbness of the tongue and slight lightheadedness during the infusion of mepivacaine.

The plasma concentrations of prilocaine were substantially less than those of mepivacaine in all instances (fig. 1). The relevant pharmacokinetic data for each drug calculated from the plasma concentration data are summarized in tables I and II. The elimination half-life data for the two drugs suggested a more rapid decrease in plasma concentrations for prilocaine. There was considerable intersubject variability in these half-life data and, at least in subject 2, the rate of elimination of mepivacaine appeared to exceed the corresponding rate for prilocaine. However, the total body clearance of prilocaine was consistently greater than that of mepivacaine and,

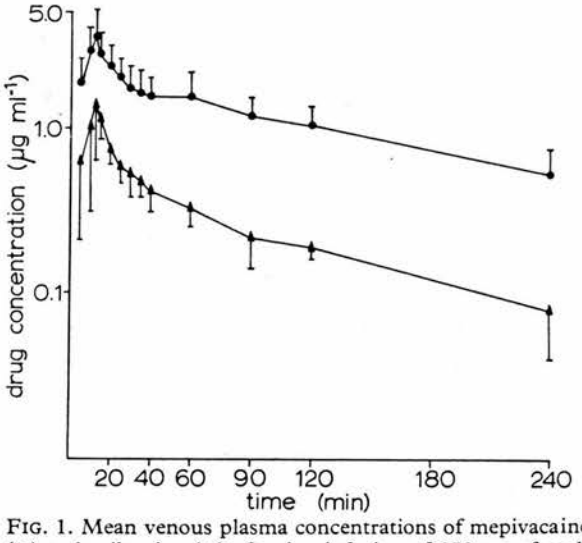

FIG. 1. Mean venous plasma concentrations of mepivacaine (●) and prilocaine (▲) after i.v. infusion of 250 mg of each drug, on separate occasions, in five male volunteers.

for each volunteer, the clearance value of prilocaine was in excess of 2 litre min^{-1}.

DISCUSSION

Local anaesthetic agents, in particular lignocaine and bupivacaine, have been subject to extensive pharmacokinetic evaluation. Attempts have been made to associate blood concentrations of these drugs with their pharmacological side-effects (Boyes et al., 1971; Reynolds, 1971b; Scott, 1975). Although early reports suggested that prilocaine was less toxic than other amide local anaesthetic agents and that plasma concentrations were substantially smaller when equal doses were administered, this drug has not been the subject of a detailed pharmacokinetic evaluation. The pharmacokinetics of mepivacaine has been reported previously in volunteers (Reynolds, 1971a; Tucker and Mather, 1975). It should be noted that the data for this drug shown in table I compare well with those reported previously by Tucker and Mather (1975). For example, they reported a mean terminal half-life of 114 min and a clearance of 0.78 litre min^{-1}, (in seven subjects) compared with the values of 125 min and 0.70 litre min^{-1} respectively in the present study.

The large clearance values for prilocaine shown in table II are of particular interest. Not only are these values approximately four times greater than those for mepivacaine but also they exceed 2 litre min^{-1} in each individual. Local anaesthetic drugs of the amide type are usually considered to be metabolized almost entirely by the liver. The mean value for hepatic blood flow in a normal population is often taken to be 1.7 litre min^{-1} (Price et al., 1960)—less than all the calculated clearance values for prilocaine in this study. This large plasma clearance of prilocaine may be explained by three possible mechanisms. First, all our volunteers may have had inordinately great hepatic blood flows. This explanation is unlikely because the clearance values for mepivacaine are very similar to those reported previously and if our volunteers were unusual their clearance values would have been greater. Second, AUC_c, which was used as the denominator in the clearance calculation (Appendix, equation 5), may have been underestimated because blood sampling was terminated at 240 min. Prilocaine taken up by tissues may have returned to the general circulation only very slowly. This is unlikely to be the complete explanation of the high calculated clearance because in every instance virtually no drug was detectable in the samples at 240 min. AUC occurring after the initial 240-min measurement period would have to equal 50–100% of the AUC during the first 4-h measurement period in order that the clearance values be calculated at values equal to or less than hepatic blood flow. Furthermore, there was no indication that the slope of the plasma concentration curve was reducing as might be expected if drug was being returned to the circulation from a storage site. Third, it is possible that prilocaine undergoes some degree of extra-hepatic metabolism. In an early pharmacological evaluation Åkerman and others (1966) reported that prilocaine could be metabolized by both lung and kidney slices from the guineapig. It has been reported that prilocaine metabolism is an anaerobic process not involving the oxidative enzymes of the liver (Geddes, 1965; Åkerman et al., 1966). It is possible that the enzymes responsible for this amide hydrolysis process are not located exclusively in the liver. Because the lungs receive the entire cardiac output, it is attractive to speculate on this organ as a possible site of extra-hepatic metabolism. It is necessary only to postulate a very low extraction and metabolism of the drug by lung tissue in order to account for clearance in excess of hepatic flow. Further detailed experiments in animals are necessary to elucidate the site and mechanism of this possible extra-hepatic metabolism. If the lung is involved, the clinical implications could be extensive. The possibility that the lung acts as a buffer between the site of injection and the systemic circulation, thereby reducing the toxicity of certain local anaesthetic drugs, would be a specific example.

It is also noteworthy that in these experiments there was little or no objective or subjective sign of toxicity observed in any of the volunteers. This might be expected with prilocaine. However, previous reports have implied that the toxicity of mepivacaine was comparable to that of lignocaine. If compared with a previous study (Scott, 1975) in which lignocaine was administered under similar conditions to a group of volunteers (including four of the five subjects in the present study), the current data would suggest that, when administered i.v., mepivacaine may be less toxic than lignocaine and that larger plasma concentrations may be tolerated before side-effects are observed. However, it should be noted that, as previously reported by Tucker and Mather (1975), the clearance of mepivacaine is less than that for lignocaine. Therefore, there will be a greater tendency for mepivacaine to accumulate following multiple injections, as might occur in obstetric analgesia.

There are certain circumstances in clinical practice in which high doses of local anaesthetic drug must be given to ensure success. In addition, certain

techniques involve injecting the drug i.v., for example Bier's block. In such instances prilocaine would appear to offer important advantages in terms of toxicity. The drug is frequently rejected because it can cause methaemoglobinaemia, but the consequences of this are seldom of clinical importance and this disadvantage is considerably outweighed by the advantage of reduced central toxicity.

APPENDIX

Pharmacokinetic analysis

The plasma concentration–time relationships, after stopping the i.v. infusion, for both prilocaine and mepivacaine were analysed using equation (1):

$$C_1 = A^1 e^{-\alpha t} + B^1 e^{-\beta t} \quad (1)$$

where C_1 = plasma concentration of the drug, α = the slope of the rapid distribution phase of the drug, β = the slope of the slower elimination phase of the drug, and A^1 and B^1 are the intercept values of the α and β phases on the plasma drug concentration axis at time (t) = 0.

The values of A^1 and B^1 were corrected for the infusion time using equations (2) and (3) (Loo and Riegelman, 1970):

$$A = \frac{\alpha\tau}{1 - e^{-\alpha\tau}} . A^1 \quad (2)$$

$$B = \frac{\beta\tau}{1 - e^{-\beta\tau}} . B^1 \quad (3)$$

where τ = the infusion time and A and B are the corrected intercept values.

The area under the plasma concentration curve (AUC) was measured (AUC_m) using the trapezoidal rule and a correction for infinite time (Gibaldi, 1977).

AUC was also calculated (AUC_c) from the relationship:

$$AUC_c = \frac{A}{\alpha} + \frac{B}{\beta} \quad (4)$$

AUC_c was used to estimate the total body clearance of each drug from the following equation (Gibaldi, 1977):

$$\text{Total clearance } (\dot{V}) = \frac{F . \text{dose}}{AUC_c} \quad (5)$$

Normally, it is assumed that F (the fraction of the drug reaching the systemic circulation) equals 1 when drugs are administered i.v.

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PHARMACOCINETIQUE ET ETUDES CLINQUES PHARMACOLOGIQUES DE LA MEPIVACAINE ET DE LA PRILOCAINE

RESUME

On a comparé la tolérance et les propriétés pharmacocinétiques de la mépivacaïne et de la prilocaïne, après infusion intraveineuse de 250 mg (0,88 et 0,97 mmol respectivement) de chacun de ces médicaments à cinq volontaires en bonne santé. Les effects secondaires ont été mineurs et ne se sont manifestés que sur deux sujets pendant l'infusion de mépivacaïne. Les concentrations de mépivacaïne dans le plasma ont été plus importantes, sur chaque sujet, que les valeurs correspondantes pour la prilocaïne. La demi-vie d'élimination de la mépivacaïne a en général été plus longue que celle applicable à la prilocaïne, alors que le coefficient d'épuration plasmatique total du corps, applicable à la prilocaïne, a été constamment plus important que le valeur correspondante applicable à la mépivacaïne. Pour chaque sujet, le coefficient d'épuration plasmatique de la prilocaïne a dépassé d'une manière substantielle le débit sanguin hépatique normal et on a donc postulé un site autre que la région hépatique pour le métabolisme de la prilocaïne.

PHARMAKOKINETISCHE UND KLINISCH-PHARMAKOLOGISCHE UNTERSUCHUNGEN MIT MEDIVACAIN UND PRILOCAIN

ZUSAMMENFASSUNG

Nach intravenöser Infusion von 250 mg (0,88 bzw. 0,97 nmol) Mepivacain und Prilocain in fünf gesunde freiwillige Versuchspersonen wurden die Toleranz und pharmakokinetischen Eigenschaften der beiden Drogen verglichen. Nebenwirkungen waren geringfügig und kamen nur bei zwei Versuchspersonen während der Mepivacaininfusion vor. Die Plasmakonzentrationen von Mepivacain waren in allen Versuchspersonen grösser als die entsprechenden Werte für Prilocain. Der Eliminationshalbwert war

allgemein grösser für Mepivacain, als für Prilocain, wohingegen die Gesamt-Prilocainclearance des Körpers konsistent höher lag, als der entsprechende Wert für Mepivacain. Bei jeder Versuchsperson überschritt die Prilocainclearance wesentlich den normalen hepatischen Blutstrom und es wurde daher ein extra-hepatischer Situs für den Prilocainstoffwechsel postuliert.

ESTUDIOS FARMACOCINETICOS Y FARMACOLOGICOS CLINICOS CON MEPIVACAINA Y PRILOCAINA

SUMARIO

La tolerancia y las propiedades farmacocinéticas de la mepivacaina y de la prilocaina fueron comparadas tras la infusión intravenosa de 250 mg (0,88 y 0,97 mmol, respectivamente) de cada droga en cinco voluntarios sanos. Los efectos colaterales fueron menores y sólo se presentaron en dos sujetos durante la infusión de mepivacaina. Las concentraciones de plasma de mepivacaina eran mayores en cada sujeto para con los valores correspondientes de la prilocaina. El período de vida media de eliminación de la mepivacaina era mayor, en general, que el de la prilocaina, mientras que la depuración total de prilocaina en el cuerpo era permanentemente mayor que el valor correspondiente de la mepivacaina. Para cada sujeto, la depuración de la prilocaina excedía de manera apreciable el flujo sanguíneo hepático normal y, por lo tanto, se postuló la existencia de un punto extra-hepático del metabolismo de la prilocaina.